

# JOURNAL OF **SWINE** HEALTH & PRODUCTION

Farm-level risk factors for detecting IAV

*Corzo CA, Morrison RB, Fitzpatrick AM, et al*

Effects of one-dose anti-GnRF vaccine  
on fertility

*Scheid IR, Oliveira FTT Jr, Borges AC, et al*

*M hyorhinis* antimicrobial susceptibility

*Jin LY, Hyoung-Joon M, Bo-Kyu K, et al*

Plasma transfer in low-birth-weight  
piglets

*Woon SY, Barton MD, Vanniasinkam T*



# Journal of Swine Health and Production

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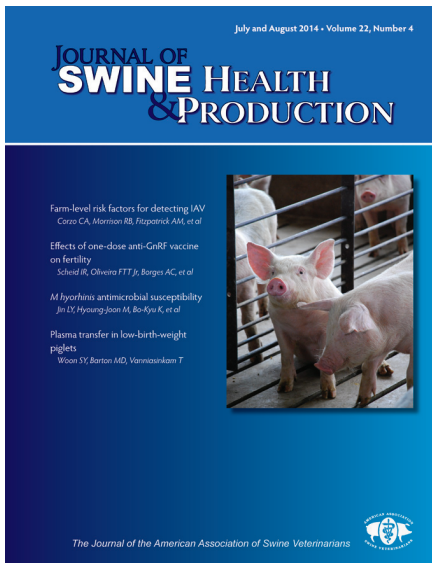
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## About the cover...



Growers in a newly filled Iowa barn

*Photo courtesy of Tina Smith*

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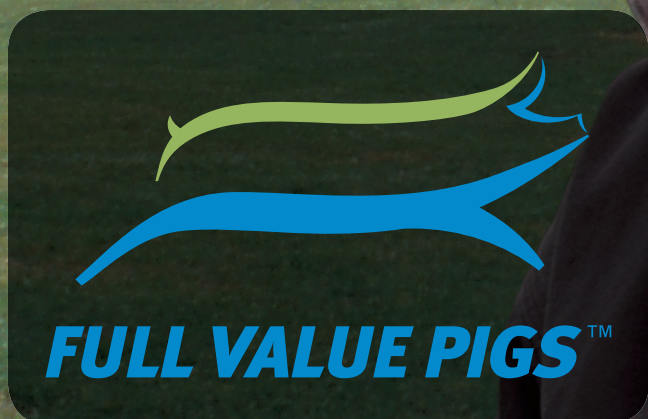
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“...future circumstances may hold challenges that we have not seen before. There may be some hard questions asked of veterinarians by farmers, state and federal animal-health officials, and even the public (both the consuming public and the non-consuming activists).”

*quoted from the Executive Director's message, page 173*

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## Lives of exemplary service

The swine veterinary profession lost two devoted colleagues this spring. While the profession will not be the same without them, we all know the profession is already different thanks to them. Drs James McKean and Scott Hurd both left their indelible marks on the swine industry, and we all benefit from their contributions.

In his president's message in the May-June 1990 issue of the AASP Newsletter, Dr McKean wrote about the unveiling of the Level III Quality Assurance Program at the World Pork Expo that year, as well as challenges the swine industry was facing with animal-rights activists. As I read his message written almost exactly 24 years ago, I am amazed by how much things have changed, and yet how much they have stayed the same.

In 1990, and again in 2014, a more comprehensive and robust version of the swine industry's quality assurance program was unveiled at the World Pork Expo. The 2014 program is intended to serve as a common industry standard and audit platform for swine producers. It will provide a more efficient, uniform process by which the industry can consolidate efforts to assure appropriate animal-welfare and food-safety standards are prevalent on farm.

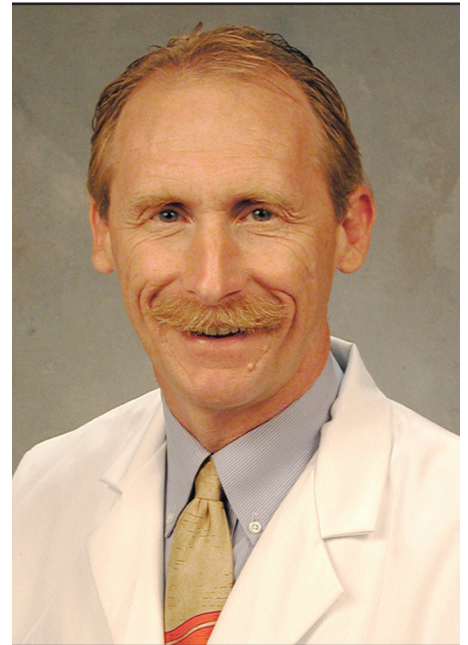


Dr McKean noted in his message that one of the key differences between the Level II and Level III programs was that Level III required on-farm residue hazards to be assessed and reviewed by a third party. I am somewhat surprised to realize we were incorporating third-party assessments nearly a quarter of a century ago, and yet the issue of third-party audits is still a "hot topic." That being said, audits are far more comprehensive today than they were in Level III, which merely required that a veterinarian assess the residue hazards on-farm.

*"Drs James McKean and Scott Hurd both left their indelible marks on the swine industry, and we all benefit from their contributions."*

In reference to challenges by animal-rights activists, Dr McKean wrote, "Swine practitioners can play a vital role in this battle by promoting continued humane treatment of swine, reducing the potential for disease through management modifications, reviewing medication needs, and encouraging residue-avoidance procedures." Jim certainly did his part in these four areas throughout his career. He was instrumental in the creation and implementation of the swine industry quality assurance programs, setting standards for both animal welfare and residue avoidance. He was also heavily involved in surveillance and management programs geared toward disease risk mitigation and pathogen elimination, which ultimately result in reduced medication usage.

Similarly, throughout his career, Dr Hurd exemplified the role of the veterinarian in his devotion to the humane treatment of swine, disease risk mitigation, judicious use of antimicrobials, and residue avoidance. Dr Hurd served in several important roles in the United States Department of Agriculture, safeguarding the health of the nation's livestock herds and the safety of the domestic food supply.



Dr Scott Hurd



Dr James McKean

*Photos Courtesy of Iowa State University College of Veterinary Medicine*

*President's message continued on page 171*

# swine influenza

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Not only did Scott work to optimize food safety through the reduction of tissue residues, he took it one step further with his risk assessments for antimicrobial-resistant bacteria. Dr Hurd was adamant that scientific information be incorporated into the risk-reward equation, that fear alone did not guide the principles of residue avoidance. In keeping with the principles of animal welfare, he helped others to understand the benefits of judicious use of antimicrobials, the risks of associated residues, and the relative risk of human illness due to antimicrobial-resistant bacteria.

One of the keys to their success was that neither Jim nor Scott was ever satisfied with the

status quo – for themselves, for their profession, or for the industries they served. Both holders of advanced degrees beyond their DVMs, these men had a thirst for knowledge and a devotion to continuous improvement. They were also committed teachers throughout their entire careers, sharing their knowledge not only with their students, but also with their colleagues, swine producers, and the general public.

Drs McKean and Hurd were both strong in their convictions. They had the education and experience that afford credibility and they were not afraid to stand up and voice their opinions (which were often facts, albeit unpopular facts). Neither was a man to shirk

responsibility or take the path of least resistance. They were proud to be veterinarians, they practiced by their oath, and the world is a better place because of them.

It is clear that these men have left a huge void in the veterinary profession and the swine industry. We must continue to build on the contributions they made to animal welfare, food safety, and education. They were excellent role models. We owe it to them to continue their legacies.

Michelle Sprague, DVM  
AASV President



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Porcine Epidemic Diarrhea (PED) is a disease that already has impacted swine operations in 28 states and Canada, killing millions of piglets. Harrisvaccines was the first company in the nation to create a PED vaccine, now available through veterinary prescription\* and anticipated to receive USDA licensure in 2014.

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## Hard questions

Swine veterinarians tend to be problem solvers by training and by nature. Given a set of facts, they want to diagnose and get to the cause, arriving at a treatment plan quickly and efficiently. They assess the efficacy of their solution and adjust as needed. Success is often evident by measures of mortality and morbidity. This approach has served the profession, our patients, and our clients well for many years. However, future circumstances may hold challenges that we have not seen before. There may be some hard questions asked of veterinarians by farmers, state and federal animal-health officials, and even the public (both the consuming public and the non-consuming activists).

Porcine epidemic diarrhea virus (PEDV) has certainly posed severe health challenges to herds throughout the United States. At the time of writing this article, we are waiting to see how the US Department of Agriculture (USDA) intends to implement the mandatory reporting of PEDV on farms and the tracking of movements (pigs, vehicles, and equipment). The details of the plan have not yet been released, but many concerns have arisen nonetheless. Concerns include

confidentiality of the data, how data will be used, and possible regulatory action that restricts the movements of pigs. The fear is that reporting and tracking will disadvantage producers but have no real impact on controlling the disease. Veterinarians are going to be asked hard questions by their clients, colleagues, and the USDA about testing protocols, PEDV diagnosis, herd management plans, pig movements, and biosecurity.

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*"...we are the health professionals with the education, the experience, and the on-farm presence to effectively advocate for the health and welfare of pigs."*

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Another issue looming in the near future is the increased role of veterinarians in the use of antimicrobials on the farm. This role will be accompanied by an increase in the accountability of the veterinarian at both state and federal levels. Many states have clarified or even increased the requirements for the writing of prescriptions, including those for extra-label drugs, as well as defining the veterinarian-client-patient relationship. The licensing of veterinarians and the regulations governing the practice of veterinary medicine occur at the state level, thus so does the enforcement. On the federal level, there are new regulations coming for the increased use of Veterinary Feed Directives, creating a new realm of paperwork and record keeping. There is no doubt that veterinarians play a vital role in the decision making on antimicrobial use in pigs. The hard questions will come in the form of documenting that role in record keeping and meeting the requirements set forth in regulations.

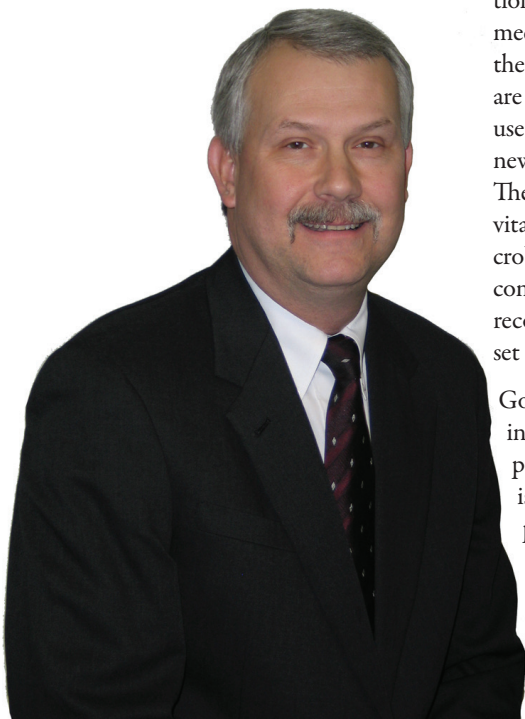
Good animal welfare is integrally woven into the day-to-day care and keeping of pigs. Increasingly this care and keeping is being challenged, sometimes by the public, the media, animal-rights activists, veterinarians, and even grocery retailers and restaurants. The latter two categories are major customers of the packers who purvey the pork produced from the raising of pigs. The pressure is mounting

on the packing industry to assure that pigs are raised and treated humanely on the farm. As time goes on, welfare audits are becoming more common on farms. This trend is not likely to abate. In conjunction with welfare audits, there are also actions being considered to ban certain production practices, such as blunt trauma euthanasia and gestation stalls. Swine veterinarians are being asked hard questions about the humaneness of these practices.

All three of these areas share a common theme centered on the role of the veterinarian on farms and with pigs. The hard questions are asked of veterinarians because we are the health professionals with the education, the experience, and the on-farm presence to effectively advocate for the health and welfare of pigs. Although it may be hard at times, we must continue to answer these questions to the best of our ability, relying on the science and art of veterinary practice to inform our answers.

All three of these areas also lead to an increasing accountability for swine veterinarians. The actions taken and the advice given by veterinarians will be open to scrutiny from several viewpoints. Increased regulatory diligence will be required for record keeping and the use of scientific data to support uses and withdrawal times of antimicrobials and other drugs. Prescriptions, Veterinary Feed Directives, and extra-label drug use will elevate the role and importance of the veterinarian working on the farm.

Over the last year and beyond, the AASV has been actively engaged in these areas. Our activity includes extensive communications with the USDA and Federal Drug Administration, as well as the National Pork Board, National Pork Producers Council, and other stakeholders. Our role in increasing knowledge among our members is our primary mission. Please consider how we can best assist in answering the hard questions that will be asked in the future.





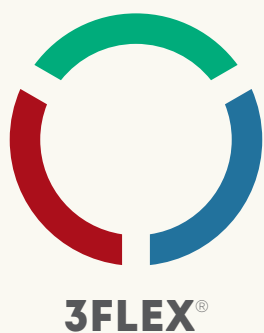
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## Author guidelines

In some of my previous editorials, I have discussed many different topics regarding scientific manuscripts and publishing. If you visit previous issues you will find messages focused on the proper use of citations<sup>1</sup> manuscript genres,<sup>2</sup> the peer-review process,<sup>3</sup> and scientific writing in general.<sup>4</sup> In this issue, I would like to discuss the journal's author guidelines. When it comes to submitting a manuscript to the *Journal of Swine Health and Production*, or any other journal for that matter, the author guidelines are your friend!

The *Journal of Swine Health and Production* follows the *American Medical Association (AMA) Manual of Style*.<sup>5</sup> The *AMA Manual of Style* is formatted as a guide for authors and editors and contains over 900 pages of very detailed information in fine print. I haven't read the manual from cover to cover, but I refer to it frequently, especially when unique situations arise – the *AMA Manual of Style* has become one of my close friends. The manual contains detailed information on formatting requirements for tables, grammar, abbreviations, how to use proprietary names, reporting of *P* values, line spacing, word spacing, layout – you get the idea. Of course it would be unreasonable for a journal to expect every submitting author to know all this information in detail – the

manual is quite thick. So *JSHAP* summarizes this information into a digestible quantity and provides the information in the form of author guidelines.

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*"When it comes to submitting a manuscript to the Journal of Swine Health and Production ..., the author guidelines are your friend!"*

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Obviously this means there will likely be some authors who will not be aware of every format detail and edits will be required. For example, when uncommon or unique formatting requirements occur within a manuscript, we help authors meet the format requirements, because not every fine detail is within the author guidelines. Once a manuscript is accepted for publication in *JSHAP*, Dr Judi Bell, our associate editor, provides guidance to authors for the necessary corrections so the manuscript will meet the requirements of the *AMA Manual of Style*. Dr Bell provides this information to authors in the form of an expository summary. The length of an expository summary can vary quite a bit and it depends on many things, such as the manuscript genre, author writing style, the number of tables and figures, and formatting errors, to name a few.

We see some common inaccuracies when it comes to authors not following *JSHAP* author guidelines. Even experienced authors can make mistakes with their submission(s) to *JSHAP*. This is likely because not every journal uses the same guidelines and format style, and some authors have their own preferences as well. However, if a manuscript is submitted with incorrect formatting, this will delay the review process. When errors are noted at the time of submission, the manuscript will be returned to the corresponding author for re-formatting before it is accepted for review. This can be frustrating for both the author and the journal staff, as it delays the entire process.

*The Journal of Swine Health and Production* reviews numerous manuscripts per year, and the most common mistakes we see are errors in the reference list and reference

numbers within the text. This is an easy mistake to avoid by simply looking at recent issues of *JSHAP* for reference formatting and examples. Another problem area arises because authors do not follow the subheading requirements for the sections of the different genres. Sometimes entire sections are missing, such as the implications section.

The format requirements for *JSHAP* rarely change; however, the author guidelines are updated frequently. We will update the guidelines if it becomes apparent that a section is unclear or perhaps a section of the *AMA Manual of Style* needs to be included in the guidelines in more detail. I recommend that corresponding authors confirm that they are working with the most current version of the guidelines.

The full guidelines are available online via the AASV Web site at <https://www.aasv.org/shap/guidelines.pdf>. In addition to the online version, a slightly abbreviated version of the author guidelines is published annually in the January-February issue of the journal. Of course, if you have questions regarding formatting, please do not hesitate to contact the journal office. We are happy to answer questions.

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Terri O'Sullivan, DVM, PhD  
Executive Editor



# Risk factors for detecting influenza A virus in growing pigs

Cesar A. Corzo, DVM, MS, PhD; Robert B. Morrison, DVM, PhD, MBA; Ann M. Fitzpatrick, DVM, MPH; Marie R. Culhane, DVM, PhD

## Summary

**Objective:** To investigate the association between certain farm-level risk factors and the presence of influenza A virus (IAV) in growing-pig farms.

**Materials and methods:** Twenty-six pig farms participated in the study. Thirty nasal swabs from growing pigs were collected per month from each farm for 12 or 24 consecutive months between 2009 and 2011. Nasal swabs were tested for IAV by real-time reverse transcriptase polymerase chain reaction. Weather stations located at every participating farm monitored temperature, relative humidity, light intensity, and wind speed and gusts. Farm-level data was

obtained through a questionnaire to assess the relationship between the presence of IAV and farm-level characteristics.

**Results:** Of the 15,630 nasal swabs collected from growing pigs, 730 (4.6%) tested positive for IAV. Of the 522 groups of growing pigs from which nasal swabs were collected, 110 groups (20.8%) had at least one positive nasal swab. Positive nasal swabs originated from 23 of the 26 participating farms. Farm-level characteristics associated with the presence of IAV included farm type (farrow-to-finish odds ratio [OR] 3.05; nursery OR 16.69), pig flow (all-in, all out OR 0.31 by barn; OR 0.35 by site), gilt source (born at breeding site, raised off-site,

and later returned OR 0.17; off-site multiplier OR 0.25), environmental temperature, and wind speed.

**Implications:** Population dynamics, eg, nursery and farrow-to-finish farms and continuous-flow management, play important roles in the epidemiology of IAV. Possible modifications to farm type and pig flow should be considered when constructing IAV control and prevention strategies.

**Keywords:** swine, influenza A virus, risk factors, meteorological conditions, weather

**Received:** August 26, 2013

**Accepted:** December 3, 2013

## Resumen - Factores de riesgo para detectar el virus de influenza A en cerdos en crecimiento

**Objetivo:** Investigar la asociación entre ciertos factores de riesgo a nivel granja y la presencia del virus A de la influenza (IAV por sus siglas en inglés) en granjas de crecimiento.

**Materiales y métodos:** Veintiséis granjas de cerdos participaron en este estudio. Mensualmente, se recolectaron 30 muestras de hisopos nasales de cerdos de crecimiento de cada granja durante 12 ó 24 meses consecutivos entre 2009 y 2011. Las muestras nasales se analizaron en busca del IAV por medio de la reacción en cadena de la polimerasa de transcripción reversa en tiempo real. Se colocaron estaciones meteorológicas en cada una de las granjas participantes para monitorear la temperatura, humedad relativa, intensidad de

luz, y velocidad de viento y ráfagas. La información a nivel de granja se obtuvo a través de un cuestionario para valorar la relación entre la presencia del IAV y las características a nivel de granja.

**Resultados:** De las 15,630 muestras nasales recolectadas de los cerdos en crecimiento, 730 (4.6%) resultaron positivas al IAV. De los 522 grupos de cerdos de crecimiento de los cuales se recolectaron las muestras, 110 grupos (20.8%) presentaron por lo menos una muestra nasal positiva. Las muestras nasales positivas procedían de 23 de las 26 granjas participantes. Las características a nivel de granja asociadas con la presencia de IAV incluyeron (ciclo completo; índice de probabilidad [OR, por sus siglas en inglés] 3.05; destete OR 16.69), flujo de cerdos (todo dentro-todo fuera OR 0.31 por edifi-

cio; OR 0.35 por sitio), origen de primerizas (granjas que crían sus propias primerizas, criadas fuera de sitio y que regresan OR 0.17; multiplicadora fuera de sitio OR 0.25), temperatura medio ambiental, y la velocidad del viento.

**Implicaciones:** La dinámica de la población, por ejemplo, las granjas de ciclo completo y manejo continuo, juegan un papel muy importante en la epidemiología del IAV. Se deberían considerar posibles modificaciones al tipo de granja y flujo de cerdos cuando se planean estrategias de prevención y control del IAV.

## Résumé - Facteurs de risque associés à la détection du virus de l'influenza A chez des porcs en croissance

**Objectif:** Étudier l'association entre certains facteurs de risque à la ferme et la présence du virus de l'influenza A (VIA) sur des fermes de porcs en croissance.

**Matériels et méthodes:** Vingt-six fermes ont participé à cette étude. Trente écouvillons nasaux par mois provenant de porc en croissance furent collectés sur chaque ferme pour 12 ou 24 mois consécutifs entre 2009 et 2011. Les écouvillons nasaux furent testés pour VIA par réaction d'amplification en

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chaîne en temps réel avec la transcriptase réverse. Des stations météorologiques étaient installées à chaque ferme participante afin de suivre la température, l'humidité relative, l'intensité de la lumière, et la vitesse des vents et des bourrasques. Des données sur la ferme furent obtenues à l'aide d'un questionnaire pour évaluer la relation entre la présence de VIA et les caractéristiques de la ferme.

**Résultats:** Sur les 15,630 écouvillons nasaux prélevés des porcs en croissance, 730 (4,6%) se sont avérés positifs pour VIA. Sur les 522 groupes de porcs en croissance à partir desquels les écouvillons nasaux furent prélevés, 110 groupes (20,8%) avaient au moins un écouvillon nasal positif. Les écouvillons nasaux positifs provenaient de 23 des 26 fermes participantes. Les caractéristiques des fermes associées à la présence de VIA incluaient le type de ferme (ratio de cote [OR] naisseur-finisser 3,05; OR pouponnière 16,69), le flot des animaux (OR tout plein-tout vide 0,31 par bâtiment; OR 0,35 par site), la source des cochettes (nées sur le site de reproduction, élevées hors-site, et retournées sur la ferme OR 0,17; multiplicateur hors-site OR 0,25), la température environnante, et la vitesse des vents.

**Implications:** Les dynamiques de populations (pouponnières et les fermes naisseur-finisser et un flot continu d'animaux) jouent des rôles importants dans l'épidémiologie du VIA. Des modifications possibles au type de ferme et au flot des animaux devraient être pris en considération lors de l'élaboration de stratégies de prévention et de limitation du VIA.

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**I**nfluenza A virus (IAV) is an RNA, single-stranded, negative-sense virus belonging to the *Orthomyxoviridae* family. The virus can infect humans and certain domestic and wild animal species, including avian, porcine, equine, canine, feline, and marine mammals. In swine, the virus is considered to play a primary role in polymicrobial respiratory-disease events.<sup>1</sup> Swine have been recognized as an important host species for IAV, since they may be potential sources for both zoonotic infections and novel viruses through reassortment.<sup>2-4</sup>

Among the different IAV subtypes, three (H1N1, H1N2, H3N2) have been circulating in the swine population during recent decades.<sup>5-7</sup> Infections in swine are

characterized by high morbidity and low mortality. Infected pigs may exhibit sneezing, coughing, lethargy, fever, anorexia, and rhinorrhea.<sup>8</sup> Infected pigs start shedding infectious viral particles through their nasal secretions 1 day after infection, and individual pigs can continue to shed for 7 days. Introduction of new viruses into pig farms may accompany infected replacement animals.<sup>5,9</sup> Pigs infected with influenza A virus can generate infectious aerosols that may play a role in regional dissemination.<sup>10</sup> However, there are gaps in our knowledge of airborne and regional transmission routes and also many unanswered questions regarding the overall epidemiology and possible modes of transmission of influenza in swine.

Risk-factor studies based on serologic data have generated valuable information regarding the epidemiology of influenza in swine. The presence of anti-influenza antibodies has been associated with high farm density, farm type, herd size, female replacement rates, pen density, uncontrolled access of people, and indoor housing.<sup>11-16</sup> However, to the authors' knowledge, there are no risk factor studies associating the presence of the virus itself with farm-level characteristics. Therefore, the objective of this study was to collect virologic and epidemiologic data to investigate whether certain farm-level risk factors are associated with the presence of IAV in growing pigs.

## Materials and methods

Procedures performed in this study were approved by the University of Minnesota Institutional Care and Use Committee.

### Study population

This study was part of a larger IAV active surveillance study conducted in the midwestern United States that started in June 2009 and ended in December 2011.<sup>17</sup> A total of 32 conveniently selected farms were enrolled, for which the primary objective was to actively monitor IAV in growing pigs over time. Six farms withdrew from the surveillance study, leaving 26 farms located in Illinois (n = 4), Indiana (n = 8), Iowa (n = 10), and Minnesota (n = 4) remaining enrolled.

### Sample collection and testing

Sample collection began once producers agreed to participate and lasted for 12 or 24 months. On each farm, nasal swabs (Star-swab II; Starplex Scientific Inc, Etobicoke,

Ontario, Canada) were collected monthly from a convenience sample of 30 growing pigs (95% confident of detecting at least one positive swab when virus prevalence is at least 10%) at approximately 10 weeks old. On pig farms where there were multiple age groups, the investigator selected the age group of pigs that was closest to 10 weeks old. Nasal-swab samples collected during the monthly visit belonged to pigs from the same age-group cohort. Swabs were individually labeled with the farm identification number, state, month, and sample number. Samples were placed in a Styrofoam container with ice packs and shipped overnight to the virology department of St Jude Children's Research Hospital (Memphis, Tennessee) for IAV testing by real-time reverse transcriptase polymerase chain reaction (RRT-PCR).<sup>17-19</sup>

### Data collection

A questionnaire (Figure 1) containing closed-ended questions was designed to capture data on farm characteristics. The survey was modeled after biosecurity questionnaires accepted by the swine industry and available through the American Association of Swine Veterinarians Production Animal Disease Risk Assessment Program (AASV PADRAP at [www.padrapp.org](http://www.padrapp.org)).

Specifically, the survey assessed farm type, regional farm density, topography surrounding the site, entrance biosecurity measures for employees and visitors, pig flow, origin of pigs, source of gilts, vaccination history, water-treatment protocols, and number of people working at the farm. The person collecting the monthly nasal swabs administered the survey to the owner or farm manager near the time of completion of the study.

### Meteorological data collection

A weather station (HOBO; Onset Computer Corporation, Bourne, Massachusetts), set up to log data every hour, was placed 20 to 30 m away from the pig barns on each of the participating farms. Meteorological data recorded included temperature (°C), relative humidity (%), sunlight intensity (watts per m<sup>2</sup>), wind direction (degrees), wind speed (km per hour), and wind-gust speed (km per hour). Data were downloaded into a portable computer from the weather station monthly on the day nasal swabs were collected.

**Figure 1:** Farm characteristics questionnaire designed to capture data from 26 pig farms in the midwestern United States for an influenza A virus (IAV) risk-factor study. The questionnaire was based on biosecurity questionnaires accepted by the swine industry, such as those available from the American Association of Swine Veterinarians Production Animal Disease Risk Assessment Program ([www.padrapp.org](http://www.padrapp.org)). The questionnaire was administered to producers from commercial farms participating in a surveillance program wherein monthly nasal swabs were collected from growing pigs for 12 to 24 months to test for IAV in an investigation of the association between certain farm-level risk factors and the presence of IAV.

## Farm Characteristics Survey

Farm Number: \_\_\_\_\_ Survey Date (MM/DD/YY): \_\_\_\_/\_\_\_\_/\_\_\_\_

### 1. Stages of production at this site

Farrow-to-finish  Farrow-to-feeder  Farrow-to-wean  Wean-to-finish  Nursery only  Gilt developer   
Nursery and finisher  Finisher only

### 2. Number of barns on-site? \_\_\_\_\_

3. Barn ventilation: Natural ventilation  Mechanical (fans and inlets)  Combination mechanical and natural

4. Topography at the site: Flat  Gentle rolling hills  Steep hills  Mountains

5. Forestation around site: No trees  Moderate forestation  Dense forestation

### 6. Pig density

Number of swine sites within 1-mile radius of this site: \_\_\_\_\_

Number of swine sites in 1- to 3- mile radius of this site: \_\_\_\_\_

Number of swine sites in 3- to 5- mile radius of this site: \_\_\_\_\_

7. Distance to nearest pig farm site: \_\_\_\_\_ miles Farm type of nearest pig farm: \_\_\_\_\_

8. Approximate number of pigs housed at nearest swine farm: Sows: \_\_\_\_ Pigs on feed: \_\_\_\_\_

9. Distance to a public road with significant (> 3 loads/week) live pig transportation: \_\_\_\_ miles

10. Origin of drinking water: Well  Lagoon  Other: \_\_\_\_\_

11. Chlorination of water: Not done  Done in response to problems  Done on a regular basis  Done continuously

12. Acidification of water: Not done  Done in response to problems  Done on a regular basis  Done continuously

13. Use of recycled lagoon water for flush or recharge? Yes  No

14. How many people work at this site? 1  2  3  4  5  6  7  8  9  10  > 10

### 15. Sanitation procedure for employees and visitors entering site

Coverall and boot change, hands are washed prior to entry  Shower in and clothes changed prior to entry

Unrestricted entry  Boot wash/disinfection prior to entry

### 16. Employee restrictions on visits to other swine production facilities:

No restrictions  Visits to other swine farms are restricted

Not applicable (Select if single owner-operator that has no employees)

17. Downtime (hours) required of employees after visiting other pig sites: \_\_\_\_\_ hours

18. Downtime (hours) required of visitors: \_\_\_\_\_ hours

19. Frequency of veterinary visits: Every \_\_\_\_\_ weeks No visits

20. On average, how many other visits does the farm receive per month: \_\_\_\_\_

21. Flow of pigs at this site: AIAO by site  AIAO by barn  AIAO by room  Continuous flow

22. Age spread (age of oldest pig in days minus age of youngest pig) of pigs: \_\_\_\_\_ days

23. How frequently (days between deliveries) are pigs delivered to this site: \_\_\_\_\_

Type of pigs delivered: \_\_\_\_\_

24. Number of breeding herds from which pigs are sourced at this site: \_\_\_\_\_

	Source 1	Source 2
Number of sows		
Number of people working at breeding herd		

25. Source of replacement gilts at these breeding herds:

- Other production system or multiplier
- Replacements born at breeding site and never moved from that site (closed herd)
- Replacements born at breeding site and moved to another site and later returned

26. Are sows vaccinated for flu? Yes  No  If vaccinated, when: \_\_\_\_\_ Number of doses: \_\_\_\_\_

Autogenous vaccine?: Yes  No

27. Are pigs on feed vaccinated for flu? Yes  No

If vaccinated, when: \_\_\_\_\_ Number of doses: \_\_\_\_\_ Autogenous vaccine?: Yes  No

28. Presence of ducks, geese or migratory birds within 1-mile radius of this site:

- Frequently (at least once per month)
- Occasionally (every 3 to 6 months)
- Rarely (less than once every 6 months to a year)
- There are no migratory birds near this site

29. Presence of feral pigs near this site:

- Frequently (at least once per month)
- Occasionally (every 3 to 6 months)
- Rarely (less than once every 6 months to a year)
- There are no feral pigs near this site

30. Presence of birds inside buildings? Often present  Occasionally present  Never

31. Insect screens are used to restrict entry of insects into buildings? Yes  No

32. Insecticides are used on exterior of buildings? Yes  No

33. Are there other animals on the farm site?

Yes  No  How many: Chickens \_\_\_\_\_ Ducks \_\_\_\_\_ Turkeys \_\_\_\_\_ Cats \_\_\_\_\_ Dogs \_\_\_\_\_ Horses \_\_\_\_\_ Cows \_\_\_\_\_

## Data analysis

For the purpose of this study, a group of pigs was defined as the sample set of 30 growing pigs selected for monthly monitoring. A group was considered positive if at least one nasal swab tested RRT-PCR-positive for IAV.

Repeated measures logistic regression with an autoregressive correlation matrix was used to assess the relationship between farm IAV status and farm-level risk factors. Farm was included in the model as a random effect. One logistic model examined the relationship between IAV status of the group and farm-level characteristics, and a second assessed the weather data.

Univariate analysis was performed to screen variables, and those with a  $P$  value  $< .25$  were considered for further analysis. A multivariable model was built by forcing all variables that met the screening criteria, and a stepwise backward elimination procedure was employed for model simplification by removing variables with

$P \geq .05$ . Farm was included in the model as a random effect to account for clustering of the groups of pigs per farm. Month was included in the model as the repeated measure under an autoregressive correlation structure matrix.  $P < .05$  was considered statistically significant in all analyses. SAS 9.2 (SAS Institute Inc, Cary, North Carolina) was used for all statistical procedures.

## Results

A total of 522 groups with a mean and median age of 13.5 and 13.0 weeks, respectively, were screened for IAV. Age ranged from 3.5 to 31.0 weeks of age. Eight farms were finishing farms, eight were wean-to-finish farms, four were farrow-to-finish farms, four were nursery-to-finisher farms, one was a nursery, and one was a gilt developer unit.

The number of visits per farm was not constant due to absence of pigs, time constraints, or farms withdrawing from the study. Of the 26 farms enrolled in the study, one was visited 25 times, 14 were visited 24 times, one was

visited 23 times, one was visited 21 times, two were visited 17 times, six were visited 12 times, and one was visited 11 times between June 2009 and December 2011. Thus, 32.1%, 24.7%, 18.3%, 15.5%, 4.6%, and 4.6% of the samples originated from groups of pigs in finisher, wean-to-finish, farrow-to-finish, nursery-finisher, nursery, and gilt developer unit farms, respectively.

At the individual level, 730 of 15,630 nasal swabs (4.7%) tested positive for IAV, whereas at the group level, 110 of 522 groups of pigs (21.1%) had at least one RRT-PCR-positive nasal swab. All but three farms had at least one IAV-positive group. The three farms with no IAV-positive groups were monitored for 12 months or less.

## Farm-level factors

For the univariate analysis, the odds of testing positive for IAV were 3.05 and 16.69 times higher for farrow-to-finish and nursery farms, respectively, than for finishing farms (Table 1). Pig farms that managed their pigs all-in, all-out (AIAO) by barn or by site,

**Table 1:** Farm-level factors univariably associated with the presence of influenza A virus in 26 commercial farms in the midwestern United States\*

<b>Risk factor</b>	<b>Odds ratio</b>	<b>95% CI</b>	<b>P†</b>
<b>Farm type</b>			
Finisher (referent)	1	NA	NA
Farrow-to-finish	3.05	1.56-5.95	< .001
Wean-to-finish	0.89	0.44-1.80	.76
Nursery	16.69	5.34-52.18	< .001
Gilt developer unit	1.11	0.31-3.94	.86
Nursery-finisher	0.78	0.33-1.82	.58
Sow vaccination for influenza	1.09	0.31-3.75	.89
No. of barns on site	1.03	0.89-1.20	.64
<b>Barn ventilation</b>			
Natural and mechanical (referent)	1	NA	NA
Natural ventilation	0.55	0.17-1.77	.32
Mechanical ventilation	1.33	0.52-3.40	.55
Topography – gentle rolling hills	0.76	0.30-1.94	.56
Absence of trees surrounding the site	0.72	0.24-2.13	.56
No. of pig farms within 1 mile	1.01	0.70-1.46	.94
Distance to closest pig farm	0.86	0.62-1.19	.37
Distance to closest road	0.89	0.71-1.10	.30
Drinking water chlorinated	1.56	0.52-4.70	.42
Drinking water acidified	1.56	0.52-4.70	.42
Recycled lagoon water for flush or recharge	1.08	0.11-9.88	.95
No. of employees at the site	1.04	0.88-1.22	.62
<b>Entrance sanitation procedure</b>			
Shower in and clothes changed (referent)	1	NA	NA
No measures	0.39	0.10-1.56	.19
Boot wash and disinfection	0.54	0.11-2.67	.45
Coverall and boot change, hands washed	0.44	0.16-1.19	.11
Employee restrictions on visits to other pig farms	0.87	0.37-2.03	.76
Downtime required for employees after visiting other pig farms	1.19	0.74-1.93	.46
Downtime required for visitors	1.12	0.67-1.86	.64
Frequency of veterinary visits	0.97	0.93-1.02	.34
<b>Pig flow</b>			
Continuous flow (referent)	1	NA	NA
All-in, all-out by barn	0.31	0.14-0.66	< .01
All-in, all-out by site	0.35	0.14-0.88	.03
No. of sow herds supplying pigs	1.51	0.59-3.82	.40
Sow herd size	1	1.0-1.0	.31
No. of workers at the sow herd	0.97	0.90-1.04	.46
Growing pigs vaccinated for influenza	1.14	0.27-4.78	.85



**Table 1:** Continued

<b>Gilt source</b>			
Born at breeding site and never moved from that site (referent)	1	NA	NA
Born at breeding site; moved to another site, later returned	0.17	0.04-0.63	< .01
Multiplier	0.25	0.09-0.68	< .01
<b>Presence of migratory birds within 1-mile radius of the site</b>			
Frequently (once per month) (referent)	1	NA	NA
Never	1.48	0.38-5.81	.57
Rarely (< once every 6 months)	2.12	0.70-6.37	.18
Occasionally (every 3 to 6 months)	1.71	0.53-5.50	.37
Presence of feral pigs near the site	1.59	0.20-12.14	.65
Presence of birds inside buildings	0.76	0.33-1.74	.53
Use of insecticides on building exterior	1.74	0.55-5.49	.34
Presence of other animals in the farm†	1.58	0.72-3.45	.24

\* Farms participated in a surveillance program wherein monthly nasal swabs were collected from growing pigs for 12 to 24 months to test for influenza A virus in an investigation of the association between certain farm-level risk factors and the presence of IAV. Farm-specific characteristics obtained through the questionnaire are described in Figure 1.

† Repeated measures logistic regression with an autoregressive correlation matrix; P < .05 considered statistically significant.

‡ Animals such as dogs and cats that are allowed entrance into the farm; not wildlife.

NA = not applicable.

compared to farms that managed pigs in a continuous flow, had lower odds of testing positive for IAV (0.31 for AIAO by barn, 0.35 for AIAO by site). Growing pigs born in sow farms in which gilts originated from an off-site facility had lower odds (0.17 if gilts were born at a breeding site, moved to another site and later returned; 0.25 if gilts originated from a multiplier) than did pigs born in sow farms in which replacement gilts were born at the breeding site and never moved from that site. The presence at the site of other animals, such as dogs and cats, was not identified as a significant risk factor for detection of IAV in pigs. Of the four variables included in the multivariable model (ie, farm type, pig flow, gilt source, and presence of other animals), only farm type remained significant.

### Meteorological factors

All measures met the multivariable model inclusion criteria (Table 2). Temperature and wind speed remained in the final multivariable model after backwards stepwise elimination. Each degree increase in temperature increased the likelihood of a group of pigs testing positive for IAV by 1.04 (95% CI, 1.01-1.07). Similarly, the likelihood of testing

positive for IAV increased 1.24 times with every km per hour increase in wind speed (95% CI, 1.08-1.43).

### Discussion

Fully understanding the ecology, evolution, and transmission of influenza A viruses requires both virological detection techniques and epidemiological investigation methods such as we have described in the present study. Although previous studies have associated farm-level characteristics with increased risk of seropositivity for IAV, to the authors' knowledge, this is the first study in which the presence of the virus in pig farms has been associated with farm-level and meteorological risk factors in swine.

The infection dynamics of the IAV are farm-type dependent. Farm type has been found as a significant risk factor according to our study reported here and in a previously published serological risk factor study.<sup>14</sup> Both studies concluded that finisher pigs were more likely to be IAV-positive when sows were on site than were finisher pigs raised on farms separated from the sow herd. It has been reported that pigs become infected at an earlier age in farrow-to-finish farms than

in finisher-only herds.<sup>20</sup> In farrow-to-finish farms, which contain all the different age groups of pigs on the same site, the virus is allowed to perpetuate due to the constant presence of susceptible individuals with waning maternal antibodies (ie, suckling piglets approaching weaning age). The existence of susceptible individuals of varying ages is absent in finishing farms that contain pigs only in the later stages of growth. Nursery farms, like farrow-to-finish farms, also have a constant influx of recently weaned pigs which provides the necessary conditions for IAV to maintain transmission between older pigs and the incoming pigs. Additionally, it has been reported that recently weaned pigs themselves can be a source of virus by introducing new viruses into recipient barns.<sup>21,22</sup> Furthermore, personnel and equipment shared between these different age groups may play a role in IAV transmission, since it is known that the virus can survive outside the swine host.<sup>23-27</sup> Since introduction of infected animals is one of the most important risk factors for new pathogen introduction into a farm,<sup>28</sup> it is not surprising that pig movement within a farm is an important factor to consider when assessing risk of IAV in pigs. There

**Table 2:** Meteorological factors univariably associated with the presence of influenza A virus in growing pigs in the midwestern United States\*

Variable	Odds ratio	95% CI	P†
Temperature (°C)	1.02	0.99-1.05	.06
Relative humidity (%)	0.96	0.93-0.99	.02
Light intensity (watts/m <sup>2</sup> )	1.01	1.00-1.01	.02
Wind direction (∅ degrees)	0.99	0.98-1.00	.11
Wind speed (km/hour)	1.17	1.02-1.34	.02
Wind gusts (km/hour)	1.13	1.02-1.25	.02

\* Farm-specific characteristics obtained through the questionnaire described in Figure 1. Meteorological data recorded by weather stations (Hobo; Onset Computer Corporation, Bourne, Massachusetts) 20-30 m from the barns.

† Repeated measures logistic regression with an autoregressive correlation matrix;  $P < .05$  considered statistically significant

are two main types of animal flows: AIAO and continuous flow. In an AIAO management system, the entire building is emptied out at one time, and then one age group of pigs enters the building, moving through the production system together. Conversely, continuous flow means that pigs are constantly entering and exiting the building, and pigs of different age groups are housed in the same building. It has been reported that AIAO pig flow has a significant positive impact on growth rate, since pigs are not being challenged with infectious agents from older pigs.<sup>28-30</sup> Our data show that pigs were less likely to test positive for IAV if they were raised under an AIAO system as analyzed by barn (OR = 0.31; 95% CI, 0.14-0.66) or by site (OR = 0.35; 95% CI, 0.15-0.88) than if they were raised under continuous-flow management. Pigs of different age groups are not exposed to one another when utilizing AIAO flow, which precludes horizontal transmission of infectious agents from older to younger pigs, a known mechanism for IAV maintenance in pig populations.<sup>5</sup>

In addition to pig flow, gilt replacement source was also associated with IAV positivity. Groups of pigs born in farms where the gilt source was an off-site facility had a lower likelihood of testing IAV-positive than did groups of pigs born in sow farms where gilts were born and raised on-site. This finding contrasts with what has been previously published, in that farms introducing replacement animals were reported to be at higher risk of being IAV-seropositive.<sup>5,14-16</sup> Furthermore, introduction of replacement animals should also maintain the circulation of IAV in the population due to the influx

of susceptible animals and new viruses.<sup>5</sup> Yet this appeared not to be the case in our study: detection of IAV in growing pigs was less frequent on farms that introduced gilts raised in off-site facilities. In today's swine farms, veterinarians are aware of the importance of controlling gilt introduction to reduce disease transmission. It will be important in future studies to determine if incoming gilts from high-health multiplier systems with no detectable IAV, or even from AIAO gilt developer units with infrequent detection of IAV, affect the number of viral introductions into the recipient farms.

Even in today's swine farms, where pigs are raised entirely indoors, weather conditions still influence the environment of the pig inside the barn (for example, low outside temperatures trigger the need to provide a heat source to increase barn temperature). Data on the relationship between environmental conditions and the presence of IAV in pigs is scarce. However, there is data regarding environment and disease transmission in other species or involving different microbiological agents. Specifically, meteorological conditions have been associated with regional dissemination of equine IAV in Australia<sup>31</sup> and with airborne detection of porcine reproductive and respiratory syndrome virus and *Mycoplasma hyopneumoniae* in pigs.<sup>32,33</sup> Our study detected an association, albeit with a measurably small effect with OR near 1.0, between two meteorological variables and the presence of IAV in groups of growing pigs. As outside temperature and wind speed increased, the likelihood of a group of pigs being infected with IAV increased. This may seem counter-intuitive, as some experimental

work with guinea pigs has shown that at high temperatures (30°C) influenza aerosol transmission ceased.<sup>34</sup> However, follow-up studies on the effects of temperature and influenza transmission showed that while aerosol transmission decreased at 30°C, direct-contact transmission was still maintained in the experimentally infected guinea pigs.<sup>34,35</sup> A reasonable interpretation for the relationship between temperature increase and presence of IAV in swine farms may be that as environmental temperature increases, the temperature of the barn increases as well, creating the need for increased air movement to reduce ambient temperature. A higher rate of air exchange in the building can be accomplished either by increasing exhaust fan speed or by lowering the curtains, increasing the entry of external air particles that may include airborne pathogens. However, it has been reported<sup>36</sup> that higher rates of air exchange in pig units have a protective effect on pneumonia lesions at slaughter, suggesting that as the concentration of housing-unit air particles decreases, respiratory lesions decrease. On the other hand, increasing wind speed may reduce external temperatures, but it is not clear what impact this has on pig-barn environmental conditions. Also unclear is the effect of wind direction on the odds of being IAV-positive. In particular, it would be interesting, and perhaps enlightening, to correlate not only wind direction but direction of the nearest farm in relationship to wind direction (eg, downwind or upwind). In this study, we recorded only the distance to the nearest farm and not the location of the nearest farm. Even though nearest farm location was not recorded, neither wind direction nor nearest neighbor nor number of pigs farms nearby were significant. Thus, directionality or predominant wind direction may be a moot point. Finally, one could speculate that at higher wind speeds, virus particles would become disrupted, and a so-called "viral cloud" could not stay intact. These associations and speculations require further investigation to deepen our understanding of the impact of meteorological conditions on disease within the barn. Until more studies are performed examining larger numbers of farms, complete risk factor analyses that include not only the farm characteristics presented here but also meteorological data, efforts at controlling IAV may best be focused on the more impactful variables of pig flow, gilt source, and farm type rather than environmental conditions and weather.

The small number of farms in this study was likely a limitation.

Farm-level risk factors identified in this study provide insights into understanding the epidemiology of influenza in swine. Virological detection coupled with risk-factor identification through epidemiological investigations such as this are encouraged as part of a global surveillance effort not only to more fully understand IAV in pigs, but also to assess the impact swine IAV may have on human health.<sup>37</sup> In addition, determining virus genetic characteristics is also encouraged in an attempt to further elucidate possible virus-level characteristics important for IAV control in swine populations. This study emphasizes the importance of population dynamics, in that certain farm-type facilities (nursery and farrow-to-finish) and pig flow (continuous flows) play a role in the epidemiology of the disease due to the constant entry of animals into a population, providing the necessary susceptible hosts for virus maintenance and increased likelihood of IAV detection. Therefore, efforts should be made to decrease IAV transmission and endemic IAV infections by managing closed populations and pig movements AIAO, which will benefit not only pigs but also possibly humans.

## Implications

- Growing pigs on farms where sows are present or where replacement gilts are raised on site are at higher risk of testing positive for IAV than pigs in finishing farms or farms that introduce replacement gilts from outside sources.
- Management practices such as AIAO may reduce the likelihood that pigs test positive for IAV.
- More research is needed to fully understand the relationships between weather and IAV in pig populations.

## Acknowledgements

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## Conflict of interest

None reported.

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\* Non-refereed reference.



# CONVERSION TABLES

## Weights and measures conversions

Weights and measures			
Common (US)	Metric	To convert	Multiply by
1 oz	28.35 g	oz to g	28.4
1 lb (16 oz)	453.59 g	lb to kg	0.45
2.2 lb	1 kg	kg to lb	2.2
1 in	2.54 cm	in to cm	2.54
0.39 in	1 cm	cm to in	0.39
1 ft (12 in)	0.31 m	ft to m	0.3
3.28 ft	1 m	m to ft	3.28
1 mi	1.6 km	mi to km	1.6
0.62 mi	1 km	km to mi	0.62
1 in <sup>2</sup>	6.45 cm <sup>2</sup>	in <sup>2</sup> to cm <sup>2</sup>	6.45
0.16 in <sup>2</sup>	1 cm <sup>2</sup>	cm <sup>2</sup> to in <sup>2</sup>	0.16
1 ft <sup>2</sup>	0.09 m <sup>2</sup>	ft <sup>2</sup> to m <sup>2</sup>	0.09
10.76 ft <sup>2</sup>	1 m <sup>2</sup>	m <sup>2</sup> to ft <sup>2</sup>	10.8
1 ft <sup>3</sup>	0.03 m <sup>3</sup>	ft <sup>3</sup> to m <sup>3</sup>	0.03
35.3 ft <sup>3</sup>	1 m <sup>3</sup>	m <sup>3</sup> to ft <sup>3</sup>	35
1 gal (128 fl oz)	3.8 L	gal to L	3.8
0.264 gal	1 L	L to gal	0.26
1 qt (32 fl oz)	946.36 mL	qt to L	0.95
33.815 fl oz	1 L	L to qt	1.1

## Temperature equivalents (approx)

°C	°F
0	32
10	50
15.5	60
16	61
18.3	65
21.1	70
23.8	75
26.6	80
28	82
29.4	85
32.2	90
38.8	102
39.4	103
40.0	104
40.5	105
41.1	106
100	212

$$^{\circ}\text{F} = (^{\circ}\text{C} \times 9/5) + 32$$

$$^{\circ}\text{C} = (^{\circ}\text{F} - 32) \times 5/9$$

## Conversion chart, kg to lb (approx)

Pig size	Kg	Lb
Birth	1.5-2.0	3.3-4.4
Weaning	3.5	7.7
	5	11
	10	22
Nursery	15	33
	20	44
	25	55
	30	66
Grower	45	99
	50	110
	60	132
Finisher	90	198
	100	220
	105	231
	110	242
	115	253
Sow	135	300
	300	661
Boar	360	794
	363	800

$$1 \text{ tonne} = 1000 \text{ kg}$$

$$1 \text{ ppm} = 0.0001\% = 1 \text{ mg/kg} = 1 \text{ g/tonne}$$

$$1 \text{ ppm} = 1 \text{ mg/L}$$

# A single dose of a commercial anti-gonadotropin releasing factor vaccine has no effect on testicular development, libido, or sperm characteristics in young boars

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## Summary

**Objectives:** To evaluate the effects of one dose of an anti-gonadotropin releasing factor (GnRF) vaccine on testicular development, sexual behavior, and sperm characteristics in young boars.

**Materials and methods:** A total of 48 pigs were equally allocated to two treatments, Controls and Immunized, with a single dose of an anti-GnRF vaccine at 16 weeks of age. Sexual behavior was evaluated 5 to 8 weeks later. Of these 48 pigs, 22 (12 Controls, 10 Immunized) underwent weekly semen collections for 14 consecutive weeks, starting 17 weeks after immunization. One week

after completion of the weekly collections, six boars per treatment underwent daily collections for 7 days. Blood for testosterone analysis was collected from seven animals per group at 0, 2, 4, 8, 12, 16, 20, 24, and 28 weeks post immunization.

**Results:** There were no statistical differences between treatments in gonad size, the sexual behavior test, qualitative and quantitative semen characteristics, sperm morphology, time to mount, ejaculation time, or serum testosterone concentrations. There was no histological evidence of an alteration in onset and development of puberty in the immunized pigs.

**Implications:** Under the conditions of this study, one dose of an anti-GnRF vaccine given to 16-week-old boars has no effect on testicular development, sexual behavior, or sperm characteristics. As final replacement-boar testing is typically conducted after 24 weeks of age, a priming dose of vaccine could be given prior to boars undergoing final testing without negative impact on testicular development and future breeding potential.

**Keywords:** swine, Improvac, anti-GnRF vaccine, fertility

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## Resumen - Una dosis única de la vacuna comercial del factor de liberación de anti-gonadotropina no tiene efecto en el desarrollo testicular, libido, o las características del esperma en machos jóvenes

**Objetivos:** Evaluar los efectos de una dosis de la vacuna de factor de liberación de antigonadotropina (GnRF) en el desarrollo testicular, conducta sexual, y características de esperma en machos jóvenes.

**Materiales y métodos:** Se distribuyó equitativamente un total de 48 cerdos a dos

tratamientos, Controles e Inmunizados, con una dosis única de la vacuna anti-GnRF en la 16 semanas de edad. Se evaluó la conducta sexual 5 a 8 semanas posteriores. De estos 48 cerdos, 22 (12 Controles, 10 Inmunizados) fueron sometidos a colecciones de semen semanales por 14 semanas consecutivas, empezando 17 semanas después de la inmunización. Una semana después de finalizar las colecciones semanales, seis machos por tratamiento fueron sometidos a colecciones diarias durante 7 días. Se colectó sangre para análisis de testosterona de siete animales por

grupo la semana 0, 2, 4, 8, 12, 16, 20, 24, y 28 post inmunización.

**Resultados:** No hubo diferencias estadísticas entre los tratamientos en tamaño de gónada, la prueba de conducta sexual, características cuantitativas y cualitativas de semen, morfología del esperma, tiempo para montar, tiempo de eyaculación, o concentraciones de testosterona en el suero. No hubo evidencia histológica de una alteración en el inicio y desarrollo de la pubertad en los cerdos inmunizados.

**Implicaciones:** Bajo las condiciones de este estudio, una dosis de una vacuna anti-GnRF aplicada a machos de 16 semanas de edad no tiene efecto en el desarrollo testicular, conducta sexual, o características del esperma. Como la prueba final al macho de reemplazo se efectúa típicamente después de la semana 24 de edad, se podría aplicar una dosis de vacuna preparatoria antes de que los machos sean sometidos a la prueba final sin un impacto negativo en el desarrollo testicular y su futuro potencial de cría.

IRS, RAS: Scheid Assessoria Agropecuária, Canela, RS, Brazil.

FTTO Jr: Zoetis, Sao Paulo, Brazil.

ACB, TFB: Universidade Federal de Uberlândia, Uberlândia, Brazil.

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Scheid IR, Oliveira FTT Jr, Borges AC, et al. A single dose of a commercial anti-gonadotropin releasing factor vaccine has no effect on testicular development, libido, or sperm characteristics in young boars. *J Swine Health Prod.* 2014;22(3):185-192.

## Résumé - Une dose unique d'un vaccin commercial anti-facteur relâchant de gonadotropine n'a aucun effet sur le développement testiculaire, la libido, ou les caractéristiques du sperme de jeunes verrats

**Objectifs:** Évaluer les effets d'une dose de vaccin anti-facteur relâchant de la gonadotropine (GnRF) sur le développement testiculaire, le comportement sexuel, et les caractéristiques du sperme de jeunes verrats.

**Matériels et méthodes:** Un total de 48 porcs ont été distribués également à deux traitements, Témoins et Immunisés, avec une dose unique d'un vaccin anti-GnRF à 16 semaines d'âge. Le comportement sexuel fut évalué 5 à 8 semaines plus tard. De ces 48 porcs, 22 (12 Témoins, 10 Immunisés) furent soumis à

une collecte hebdomadaire de semence pendant 14 semaines consécutives, débutant 17 semaines après l'immunisation. Une semaine après avoir terminé les collectes hebdomadaires, six verrats par groupe de traitement furent soumis à une collecte quotidienne pendant 7 jours. Du sang pour dosage de la testostérone fut prélevé de sept animaux par groupe à 0, 2, 4, 8, 12, 16, 20, 24, et 28 semaines post-immunisation.

**Résultats:** Il n'y avait aucune différence significative entre les groupes de traitement en ce qui a trait à la taille des gonades, le test de comportement sexuel, les caractéristiques qualitatives et quantitatives de la semence, la morphologie du sperme, le temps de la monte, le temps d'éjaculation, ou les concen-

trations de testostérone sérique. Il n'y avait pas d'évidence histologique d'une modification dans le début et le développement de la puberté chez les porcs immunisés.

**Implications:** Dans les conditions de la présente étude, une dose d'un vaccin anti-GnRF donné à des verrats âgés de 16 semaines n'avait aucun effet sur le développement testiculaire, le comportement sexuel, ou les caractéristiques du sperme. Étant donné que les épreuves finales sur les verrats de remplacement sont effectuées après 24 semaines d'âge, une dose d'amorce de vaccin pourrait être donnée préalablement aux épreuves finales sur les verrats sans impact négatif sur le développement testiculaire et le potentiel reproducteur futur.

Immunization against gonadotrophin releasing factor (GnRF), producing an "immunological" castration, is an increasingly used alternative to physical castration of young piglets, controlling boar taint while maintaining most of the production efficiencies associated with entire males.<sup>1</sup> While this process uses the immune system to stimulate production of specific anti-GnRF antibodies, it is not classified as a "vaccine" in some countries and is thus frequently referred to as an "immunological product." The mode of action is nevertheless that of a classical vaccine, and in this report the term vaccine is used for simplicity. A commercial anti-GnRF vaccine is now available in many countries from the same manufacturer under several trade names (Improvac, Improvest, Vivax, Innosure), hereafter referred to as Improvac (Zoetis, Florham Park, New Jersey). The immunizing antigen in Improvac comprises a synthetic analogue of endogenous mammalian GnRF conjugated to a carrier protein. The result is a large protein molecule that has no intrinsic hormonal activity and is foreign to the immune system.<sup>2</sup> Formulated with an appropriate adjuvant, it stimulates the immune system, after two doses, to transiently produce high concentrations of circulating antibodies that can bind and inhibit the action of natural GnRF.<sup>1</sup>

To achieve effective suppression of testes function and clearance of boar taint, two doses must be given at least 4 weeks apart.<sup>1,3-5</sup> The first dose serves to prime the immune system and results in only a small increase in detectable circulating

anti-GnRF antibodies<sup>3-5</sup> with, at the time of the second dose, no detectable effect on circulating testosterone concentrations<sup>1,3-5</sup> or testes growth.<sup>1</sup> After the second dose, there is a strong antibody response that results in temporary suppression of testicular function.<sup>1,3-5</sup>

The procedure may be used on male pigs that fail the selection procedure for breeding boars, thus controlling boar taint and allowing their use for human consumption. However, the minimum time from first dose to slaughter is typically 8 weeks; thus, rejected breeding boars would need to be retained for at least 8 weeks after rejection (ie, after the first immunization) before they could be sold free of boar taint. Giving the first dose prior to selection testing would allow the second dose to be given at the time of the rejection decision, thus potentially saving 4 or more weeks. While the available data suggest that the first dose has no or minimal physiological effect,<sup>1,3-6</sup> and thus no impact on subsequent breeding performance, specific detailed studies are lacking. The objective of the present study was to test, for the first time to the authors' knowledge, whether a single priming dose of the anti-GnRF vaccine, Improvac, has effects on testicular development, libido, or sperm characteristics in young boars.

## Materials and methods

This study and all procedures used in the study were conducted in compliance with the Brazilian regulatory guidelines for the ethical use of animals and animal welfare.<sup>7</sup>

## Animals, housing, and feeding

Forty-eight healthy entire male pigs of a commercial Landrace × Large White genotype were selected for the trial at 14 weeks of age from a commercial swine farm in Brazil and transferred to the research facility. All animals were born in the same week, weaned on the same day, and identified with individually numbered ear tags. From 15 weeks of age, the pigs were housed in individual pens with a minimum space of 8.8 m<sup>2</sup>. Floors were partially slatted and the barn sides were open with closable curtains. Between 15 and 20 weeks of age, the animals were fed ad libitum with a commercial grower-finisher ration (crude protein 16%). From 21 weeks of age (5 weeks after immunization), the animals received 2.0 to 2.5 kg per day of a commercial boar ration (crude protein 15%), fed once a day. Body condition was evaluated each time the pigs were weighed, and the amount of feed adjusted when necessary. The animals had free access to water via nipple drinkers.

## Experimental design

At 16 weeks of age, using randomly generated numbers, the pigs were allocated into two equal groups: 24 Controls and 24 Immunized. The individual animal represented the experimental unit. At 16 weeks of age, the Control group were injected subcutaneously behind the left ear with 2 mL of non-pyrogenic sterile saline, and the Immunized group were similarly injected with 2 mL of the anti-GnRF vaccine (Improvac batch 009/08; Zoetis, Florham Park, New Jersey). The batch of Improvac

used was a full commercial batch produced under Good Manufacturing Practice (GMP) conditions, which require that every batch released must pass a full quality assessment, including potency. Personnel who were not directly involved in the collection of experimental data delivered treatments, while personnel designated to carry out the experimental observations and data collection were blind to treatment during the experimental period.

Because of space and labor restrictions and the fact that this study had multiple components, some of which were mutually incompatible, the 24 available animals per treatment were randomly shared among the components. A schedule of events for the study is shown in Figure 1. At the start of the trial, a total of 14 animals (seven per treatment) were selected by random number generation for assessment of serum testosterone at various time points during the study. Blood was collected from these animals at the following time points: treatment day (16 weeks of age), 2 and 4 weeks after treatment, and then every 4 weeks until 44 weeks of age. The same animals were used in all collections, and these animals were not used for histological assessment of the testes structure. Approximately 10 mL of blood was collected by jugular venipuncture on each occasion. Serum was obtained and stored at -20°C until used for testosterone analysis. Also at the start of the study, 10 animals per treatment were randomly allocated to be castrated, under general anesthesia, to enable the testes and epididymides to be weighed and the testes to be assessed histologically. Three animals per treatment were castrated at each of 4 and 13 weeks post treatment, and a further four per treatment were castrated at 31 weeks post treatment. All 24 pigs per treatment were individually weighed at selection (14 weeks of age) and at immunization (16 weeks of age). All available pigs from the initial 24 per treatment were again weighed 4, 13, 24, and 31 weeks after immunization. At each of these times, the width of the scrotum at its maximum width was also measured with a pair of engineering calipers.

### Testosterone analysis

Quantification of testosterone was performed using liquid chromatography with tandem mass spectrometric detection. This method was chosen instead of one of the many radio-immunoassay kits available because numerous

studies have proven the liquid chromatography-mass spectrometric (LC-MS) method to be a more reliable method of determining serum testosterone concentration.<sup>8,9</sup> The LC-MS method has been validated for pig serum consistent with the current Federal Drug Administration (FDA) Guidelines for Bioanalytical Methods Validation.<sup>9</sup> Briefly, testosterone was extracted from porcine serum using a 96-well automated liquid-to-liquid extraction at alkaline pH with ethyl acetate. Before extraction, isotope-labeled testosterone was added as an internal standard (testosterone-d<sub>3</sub>). The organic layer was collected, transferred to a new 96-well plate, and evaporated to dryness. The residue was reconstituted with a 55% acetonitrile, 45% purified water, 0.1% formic acid mobile phase and injected into a liquid chromatography-mass spectrometer using a Betasil C18 column (Keystone Scientific Inc, Bellefonte, Pennsylvania). The lower and upper limits of quantification were 0.35 and 69.4 nmol per L, respectively. Testosterone-free porcine serum samples collected from castrated boars were used as “blank” reference samples and stored under the same conditions as the study samples. Each assay included blank biological matrix and a series of six pre-prepared calibration standards, together with three in-house prepared quality control (QC) samples within the assay’s range of quantification. The mean intra- and inter-assay bias of the QC samples was < 15.0%, which is consistent with FDA guidelines.<sup>10</sup>

### Evaluation of sexual behavior

Sexual-behavior evaluations were performed when the animals were 21 to 24 weeks of age (5 and 8 weeks after treatment) by exposure of the boars to estrous gilts. Ten gilts, approximately 5 to 6 months of age, were housed as a group in the same building as the males. Thirty-eight boars (19 animals per treatment) underwent sexual-behavior evaluation. Over a 15-minute period, each boar was individually exposed to an estrous female of compatible body size in a 2 × 3-m area. The following parameters were recorded: number of pigs mounting on the first presentation to an estrous female, number of incorrect mounting attempts until a correct mount was made, and time from entering the pen until a correct mount was made. The test was concluded when the male correctly mounted. Intromission and mating were not allowed.

### Semen collection and evaluation

After the sexual-behavior evaluation was concluded, 22 boars (12 Controls and 10 Immunized) were trained for semen collection on a fixed dummy using the gloved-hand method.<sup>11</sup> The boar was considered trained when it performed the mount within 10 minutes after being presented to the dummy and allowed the total ejaculate to be collected. Over 14 consecutive weeks, from week 17 after treatment (33 weeks of age) onwards, the trained boars were subjected to semen collection at 7-day intervals. One week after completion of the 14 weekly collections, six boars per treatment underwent an intensive daily semen collection for 7 days. The same person performed all semen collections. The times between entering the collection room and an effective mount on the dummy and the ejaculation time were recorded. The quantitative and qualitative characteristics of the ejaculate were evaluated for all collections. The liquid and gel fractions of the ejaculate were separated at the time of collection using a filter adapted to the collection vial. The liquid volumes, weight of the gel fraction, sperm motility, sperm concentration, total number of spermatozoa, and sperm morphology were recorded. Sperm morphology was evaluated in all ejaculates collected during the weekly collection phase, and from ejaculates collected on days 1, 4, and 7 of the daily collection phase. The same person performed all semen examinations.

Volume of the liquid fraction was determined by weighing the liquid fraction of the ejaculate and calculating the volume, assuming a semen density of 1 g per mL. Sperm motility was determined by clear field microscopy at 100× magnification, evaluating the percentage of motile spermatozoa in a semen drop between a glass slide and cover slip previously heated to 37°C. Five to six fields per slide were evaluated. Motility evaluation was repeated three times for each ejaculate, and the best result obtained was then recorded.

Sperm concentration was determined in a hemocytometer chamber (Neubauer) and the result expressed in number of cells per mm<sup>3</sup> semen. Total number of cells in the ejaculate was obtained by multiplying the sperm concentration by the ejaculate volume. Sperm morphology was examined by phase contrast microscopy at 1000× magnification in a wet preparation, with semen fixed in formol citrate solution. The semen samples were prepared immediately after ejaculate collection and examined on the same day. In each ejaculate, 200 cells were

**Figure 1:** Timeline for the 31-week duration of a study to evaluate the effects of one dose of an anti-gonadotropin releasing factor vaccine on testicular development, sexual behavior (19 boars/treatment), and sperm characteristics in young boars from a commercial facility. At 16 weeks of age, the Control group (n = 24) were injected subcutaneously behind the left ear with a single 2-mL dose of non-pyrogenic sterile saline, and the Immunized group (n = 24) were similarly injected with 2 mL of anti-GnRF vaccine (Improvac; Zoetis, Florham Park, New Jersey). Shading indicates the assessments performed each study week. Individual body weight and scrotal width measurements were made on all available animals at each time point. Blood samples were collected from seven pre-selected pigs per treatment. Semen was evaluated on 12 Control and 10 Immunized pigs at each time point indicated, with the exception of the seven daily collections in study week 31 when six pigs per treatment were assessed. Three pigs (\*) or four pigs (†) per treatment were designated for castration for histological assessment of the testes.

Study week	Age (weeks)	Weighed	Scrotal width measurement	Blood sample	Castration	Semen evaluation	Sexual behavior
0	16	Yes	Yes	Yes	No	No	No
2	18	No	No	Yes	No	No	No
4	20	Yes	Yes	Yes	Yes*	No	No
5	21	No	No	No	No	No	Yes
7	23	No	No	No	No	No	No
8	24	No	No	Yes	No	No	Yes
12	28	No	No	Yes	No	No	No
13	29	Yes	Yes	No	Yes*	No	No
16	32	No	No	Yes	No	No	No
17	33	No	No	No	No	Yes	No
18	34	No	No	No	No	Yes	No
19	35	No	No	No	No	Yes	No
20	36	No	No	Yes	No	Yes	No
21	37	No	No	No	No	Yes	No
22	38	No	No	No	No	Yes	No
23	39	No	No	No	No	Yes	No
24	40	Yes	Yes	Yes	No	Yes	No
25	41	No	No	No	No	Yes	No
26	42	No	No	No	No	Yes	No
27	43	No	No	No	No	Yes	No
28	44	No	No	Yes	No	Yes	No
29	45	No	No	No	No	Yes	No
30	46	No	No	No	No	Yes	No
31	47	Yes	Yes	No	Yes†	Yes (daily)	No

evaluated. Alterations in sperm morphology were classified according to the spermatozoa anatomic site where they occurred. Primary and secondary acrosome defects, abnormal head and neck, mid-piece defects, and abnormal tails, as well as proximal and distal droplets, were recorded.

### Histological evaluation

The pigs selected for castration were sedated with acepromazine at 0.2 mg per kg body-weight intramuscularly (Lab Vetnil, Sao Paulo, Brazil) and anesthetized with Zoletil

50 (a combination of tiletamine hydrochloride and zolazepam hydrochloride; Virbac, Carros Cedex, France) at 3 to 5 mg per kg body weight intramuscularly. The testes were separated from the epididymides immediately after castration, and the testes and epididymides were weighed separately. Samples (approximately 2 × 2 × 1 cm) were collected from the capitata extremity, the mid-third, and the caudal extremity of the left testicle of each animal and immediately fixed in Bouin's solution for 24 hours. The samples were embedded in paraffin, and

6-μ sections were cut and stained with hematoxylin and eosin. Each section was examined at 100× power to visually assess seminiferous tubule size, degree of luminal formation, type of sperm cells present, and phase of testicular development.

### Data analysis

Data was analyzed using the SAS statistical package release 9.1.3 (SAS Institute Inc, Cary, North Carolina). A one-way ANOVA F-test was used to determine if there was an initial body weight difference between



the two treatments when the pigs were weighed at 3 to 4 weeks of age. Analysis of covariance was used to test for differences in body weight at 26, 90, 168, and 214 days of age, using the initial weight as covariable. Testosterone data were analyzed on log-transformed data by ANOVA F-test with interaction between treatment and time. Treatment and time were considered fixed effects, with animals as a random effect. Pairwise comparisons with time were tested using the Tukey-Kramer test. Scrotal width, testes weight, and epididymides weight were also analyzed using an ANOVA F-test, with pairwise comparisons using the Bonferroni test. The chi-square test and Fisher's exact test were used to analyze the sexual-behavior information using categories of one or two presentations: zero and at least one for the number of mounts, and 1 minute and  $\geq 2$  minutes for the time to mount. Except for motility, a repeated measures ANOVA F-test was used for semen data with interaction between treatment and time. Treatment and time were considered fixed effects with animals as a random effect. Because there was no variability in the motility data, an ANOVA test was not used and only descriptive statistics were used for motility. For all tests,  $P < .05$  was considered statistically significant. The histological assessments were qualitative only, and no statistical evaluations were performed on the histological observations.

## Results

There were no statistically significant differences between treatments for any of the testicular, sexual-behavior, or ejaculate parameters. Body weight did not differ between treatment groups at the start of the study (16 weeks of age): 61.9 kg versus 61.5 kg for Controls and Immunized, respectively. Body weights of the Control and Immunized groups did not differ at the other time points in the study: 83.9 kg versus 84.3 kg, 133.9 kg versus 133.9 kg, 181.7 kg versus 183.6 kg, and 198.4 versus 202.0 kg for Controls and Immunized, respectively, at 4, 13, 24, and 31 weeks after immunization (20, 29, 40, and 47 weeks of age).

In situ maximum scrotal width along with the weight of the testes and epididymides did not differ between treatments at all time points assessed and are presented in Table 1. The only differences in scrotal width, testes, or epididymides weight were the increase in these parameters with time as the pigs in both groups matured. As expected for a hormone

that is secreted episodically, the testosterone concentrations were highly variable across time, but did not differ ( $P > .05$ ) between treatments at any time point (Table 2).

There were no statistically significant differences between treatments for any sexual-behavior parameter. All pigs mounted the estrous female when presented, with 15 of the 19 Control pigs (79%) and 19 of the 19 Immunized pigs (100%) mounting on their first presentation ( $P = .11$ ). The number of incorrect mounts before a correct mount was achieved also did not differ between treatments, with 12 of the 19 Control pigs (63%) and eight of the 19 Immunized pigs (42%) mounting on the first attempt ( $P = .33$ ). Eight of the 19 Control pigs (47.0%) pigs and seven of the 19 Immunized pigs (37%) mounted within the first minute of exposure to an estrus female ( $P = .74$ ). The maximum time to perform a correct mount was 7 minutes, and no pigs exceeded four incorrect mounting attempts. For the weekly semen collections, there were no significant differences between the groups in the quantitative or qualitative characteristics of semen (Table 3). All values were within expected ranges. No parameter in either treatment showed any deterioration over the course of the 14 weeks of weekly collections.

During the week when semen was collected daily, there was a gradual deterioration in many of the parameters assessed. However, the time-dependent decline in parameter values occurred equally across both treatments (Table 4). The deterioration in gel weight, liquid fraction volume, sperm concentration, total number of sperm per ejaculate, and number of abnormal cells was statistically significant in both treatments ( $P < .05$ ) when the results of the first and last daily collections were compared. There were no between-treatment statistical differences for any parameter during the period of daily collections.

Visually, there was no qualitative histological evidence of any alteration in the onset and development of puberty in the Immunized group compared to the Controls. Qualitative histological examination revealed normal development of the seminiferous parenchyma and support structures, including Leydig cells and seminiferous tubules, in the two treatments. Tubule transformation and spermatogenesis did not differ visually between the two groups. In the youngest pigs castrated (4 weeks after treatment;

20 weeks of age), testicular development in both groups was in the pre-pubertal stage (luminal formation, incipient spermatogenesis with presence of spermatids). By 29 weeks of age (13 weeks after treatment), both groups had evolved to the pubertal stage (complete spermatogenesis and spermatozoa in the lumen of the tubules, complete luminal formation). By 47 weeks of age (31 weeks after treatment), the two groups were in the post-pubertal stage (increased numbers of spermatocytes and spermatids, adequate yield of spermatogenesis). The presence, structure, and number of Leydig cells did not differ morphologically between the treatment groups at the three ages.

## Discussion

In countries where immunization against GnRF has been used commercially, there have been many expressions of interest to use the procedure on breeder boars that fail the selection process. This would allow such animals to be sold free of boar taint and allow their use for human consumption. However, the minimum time from first dose to slaughter is typically 8 weeks, thus reject breeding boars would have to be kept for at least 8 weeks after rejection before they could be sold free of boar taint. If the first dose could be given prior to final selection testing, the second dose could be given at the time of the rejection decision, potentially saving 4 or more weeks. While the available data suggest that the first dose has minimal physiological effects on testes function,<sup>1,3-5</sup> and should have no impact on subsequent breeding performance, specific detailed studies were lacking. Such information arises from very limited observations made at the time the second dose is given, generally 4 to 6 weeks after the first dose, at 18 to 22 weeks of age.<sup>1,3</sup>

The results of the present study demonstrate that a single dose of Improvac given at approximately 16 weeks of age has no detectable effect on testes development, sexual behavior, or sperm characteristics of young boars. Giving the initial dose earlier than at 16 weeks of age (for example, at 10 to 12 weeks of age) is unlikely to alter the outcomes of the current experiment. Research data on file with Zoetis shows that there was no effect of an initial dose given at 4 weeks of age on testosterone concentrations or testes width (growth) at 19 weeks of age, when the second dose was administered (written communication; Professor Frank

**Table 1:** Mean (standard deviation) in situ scrotal width (cm) at maximum width and, for the subsets of castrated pigs, trimmed testes and epididymides weights (g) in Controls and Immunized pigs\*

Week of study	Age of pigs (weeks)	Scrotal width (cm)		Testes weight (g)		Epididymides weight (g)	
		Control	Immunized	Control	Immunized	Control	Immunized
0	16	7.79 (0.67)	7.67 (0.64)	ND	ND	ND	ND
4	20†	10.12 (0.97)	9.97 (0.64)	246.86 (84.21)†	265.94 (26.07)†	57.28 (7.39)†	57.65 (6.88)†
13	29†	14.12 (0.68)	14.03 (0.84)	628.33 (73.36)†	571.67 (65.65)†	135.67 (11.93)†	140.67 (14.74)†
24	40	15.29 (0.97)	14.96 (0.97)	ND	ND	ND	ND
31	47‡	15.68 (0.97)	15.84 (1.43)	782.00 (89.60)‡	690.25 (121.05)‡	222.50 (21.81)‡	197.75 (19.57)‡

\* Study and pigs described in Figure 1. Scrotal width measurements were made on 24 pigs per treatment at 16 and 20 weeks of age, 21 per treatment at 29 weeks of age, and 18 per treatment at 40 and 47 weeks of age. There were no statistical differences between treatments ( $P > .05$ ) for any parameter at any time point (ANOVA F-test; pairwise comparisons tested with the Bonferroni test).

† Three pigs per treatment castrated.

‡ Four pigs per treatment castrated.

ND = not determined: no pigs castrated.

**Table 2:** Mean (standard deviation) serum testosterone concentration at various time points in seven Control pigs and seven Immunized pigs\*

Week of study	Age of pigs (weeks)	Serum testosterone concentration nmol/L	
		Control	Immunized
0	16	19.6 (14.4)	14.7 (10.7)
2	18	8.4 (3.3)	4.5 (4.0)
4	20	16.6 (15.6)	4.6 (2.9)
8	24	5.4 (2.2)	4.5 (7.0)
12	28	13.5 (5.7)	17.6 (12.8)
16	32	18.9 (9.5)	24.7 (15.7)
20	36	4.1 (3.3)	5.2 (2.8)
24	40	11.2 (5.0)	19.2 (12.0)
28	44	3.0 (1.4)	4.3 (4.9)
Overall mean		11.3 (10.0)	11.5 (11.4)

\* Pigs and study described in Figure 1. There were no statistical differences ( $P > .05$ ) between treatments for testosterone concentration at any time point (ANOVA F-test; pairwise comparisons tested with the Tukey-Kramer test).

Dunshea, Chair of Agriculture, University of Melbourne, Melbourne, Victoria, Australia; 1996).

A limitation of this study is that a positive control (ie, receiving two full doses of Improvac) was not included. Thus, it is legitimate to ask whether the specific batch of Improvac was indeed biologically active and whether the immune system was actually primed. The batch of Improvac used was a full commercial batch produced under GMP conditions, which require that every batch released must pass a full quality assessment, including

potency. In addition, this batch performed satisfactorily in the field in Brazil (e-mail communication; Dr Fabio Teixeira, Technical Manager, Zoetis, Brazil), where on-line assessment of reduced testes size is routinely performed in all abattoirs. There were no reports of a lack of efficacy from these plants during the time that this batch was used in the field. Thus it is reasonable to conclude that the one dose used in this study would have in fact adequately primed the immune system with no detectable deleterious effects on testes development or sexual behavior.

There is a wealth of data available on the effects of immunization with two doses of Improvac on growth performance, boar taint, testes function, and sexual behavior. Several reports have demonstrated that immunization with two doses is efficient in eliminating boar taint and, compared with physical castrates, results in better growth performance and carcass characteristics.<sup>1,12-15</sup> In addition, as the immunized boar's testes function is temporarily inhibited, concentrations of testosterone and other sexual hormones are suppressed. As a consequence, concentrations of androstene and skatole in the subcutaneous fat are suppressed.<sup>1,4,6,16</sup> Consistent with a suppression of testes function, other studies have shown that the animals' sexual and aggressive behavior is suppressed compared to that in physical castrates.<sup>4,17,18</sup>

Administration of the first dose of Improvac at approximately 16 weeks of age coincides with development of puberty in boars at 13 to 20 weeks of age.<sup>19,20</sup> This period is characterized by rapid growth of the testes and by proliferation of Sertoli cells and establishment of spermatogenesis, which leads to the presence of free sperm in the lumina of the seminiferous tubules. Follicle stimulating hormone, luteinizing hormone, and testosterone all play fundamental roles in regulating this process. Serum testosterone concentrations reach a maximum during puberty, followed by a slight decrease before stabilizing.<sup>21</sup> Full sexual maturity generally takes place at approximately 8 months of age.

In the present study, various measures of testes function, testes morphology, and semen characteristics were made from 16 weeks of age (the middle of the pubertal period when the first dose of Improvac was administered) until 47 weeks of age. Thus, it was possible to monitor the effects of a single dose on development of the testes during the transient stage of puberty until the post-pubertal stage when sexual and reproductive maturity were reached. The results obtained indicate that, under these experimental conditions, administration of a single dose of Improvac did not affect testes development or function. At all ages that were evaluated, the size of the gonads, both the *in situ* measurements and the weight of testes and epididymides, did not differ ( $P > .05$ ) between the Immunized pigs and the Controls that received the placebo.

The growth of testes and epididymides was more intense in early puberty, between 16 and 20 weeks of age, as previously described in swine.<sup>19</sup> In boars 29 and 47 weeks of age, the mean weights of the testes (pair) in the control and treated groups were not statistically different and were similar to those observed by Silva.<sup>19</sup> The administration of a single dose of Improvac did not influence development and maturation of the testes structure, as determined by morphology and morphometry observations of the testes. Performance in the sexual behavior tests did not differ between males in the two treatment groups. The sexual-behavior observations reported here are consistent with those reported by Ferreira et al.<sup>22</sup>

In this study, semen collection was started at 33 weeks of age, coinciding with a common

age at which semen donors are introduced to regular semen collections in commercial artificial insemination centers.<sup>23</sup> There was no difference in the results of quantitative and qualitative characteristics of semen between the treatment groups: all parameters were within the physiological standards of sperm production for swine.<sup>21</sup> Finally, there was no difference in the serum testosterone concentrations of vaccinated and non-vaccinated pigs, indicating that a single dose of Improvac did not influence the serum profile of this hormone. A large variation in the testosterone concentration was observed throughout the study, which can be attributed to the episodic characteristic of testosterone release.

The detailed findings on testicular development, sexual behavior, and semen

**Table 3:** Mean (standard deviation) across the 14 weeks of weekly semen collections for measures of sexual behavior and semen assessment in 12 Control pigs and 10 Immunized pigs\*

	Control	Immunized
Time to mount (min)	3.44 (2.89)	2.61 (1.81)
Ejaculation time (min)	6.74 (2.35)	6.64 (2.26)
Gel weight (g)	49.2 (22.1)	56.2 (24.6)
Liquid fraction of ejaculate (mL)	233.5 (82.0)	257.0 (77.3)
Sperm concentration ( $10^6/\text{mm}^3$ )	0.28 (0.06)	0.26 (0.03)
Total number cells ( $\times 10^9$ )	63.1 (21.0)	66.8 (19.8)
Abnormal cells (%)	5.56 (2.70)	7.51 (5.08)
Motility (%)	81.1 (5.8)	77.5 (8.7)

\* Pigs and study described in Figure 1. With the exception of sperm motility, there were no statistical differences between treatments ( $P > .05$ ) in any parameter (repeated-measures ANOVA F-test). As there was no variability in the motility data, only descriptive statistics were used for this parameter.

**Table 4:** Mean (standard deviation) across the 7 days of daily semen collections and on the first and last days of the week of daily collections for measures of sexual behavior and semen assessment in six Control pigs and six Immunized pigs\*

	Mean for 7 days		Day 1 mean		Day 7 mean	
	Control	Immunized	Control	Immunized	Control	Immunized
Time to mount (min)	2.88 (1.97)	3.50 (2.98)	2.50 (1.97)	3.33 (3.01)	3.33 (0.52)	3.33 (0.82)
Ejaculation time (min)	6.36 (1.91)	6.48 (2.67)	7.50 (1.64)	7.67 (4.18)	6.67 (1.03)	4.50 (1.05)
Gel weight (g)	45.26 (21.5)	44.8 (29.4)	52.33 (9.58)	71.00 (52.49)	28.50 (11.41)	25.50 (9.93)
Liquid fraction of ejaculate (mL)	215.9 (97.9)	193.1 (84.0)	282.83 (62.60)	299.33 (129.23)	135.83 (60.38)	115.33 (32.32)
Sperm concentration ( $10^6/\text{mm}^3$ )	0.22 (0.07)	0.21 (0.06)	0.318 (0.06)	0.313 (0.07)	0.156 (0.02)	0.159 (0.02)
Total number cells ( $\times 10^9$ )	49.0 (32.2)	43.2 (25.8)	90.46 (30.18)	87.75 (24.97)	20.24 (6.33)	18.73 (7.28)
Abnormal cells (%)	10.58 (6.68)	15.17 (10.00)	5.00 (1.87)	9.75 (9.22)	17.58 (6.10)	21.58 (7.49)

\* Pigs and study described in Figure 1. There were no statistical differences between treatments ( $P > .05$ ) in any parameter (repeated-measures ANOVA F-test).

characteristics, reported here for the first time (to the knowledge of the authors), are to be expected and are fully consistent with the limited reports of similar testes function at the time of the second dose of the vaccine.<sup>1,3-5</sup> Because the first dose serves only to prime the immune system, possible replacement breeding boars could be given a priming dose of Improvac with confidence that there will be no deleterious short- or long-term effects on breeding performance.

## Implications

- Under the conditions of this study, administration of a single dose of the anti-GnRF vaccine, Improvac, to intact male pigs at 16 weeks of age has no effect on testicular development, sexual behavior, or sperm characteristics over the subsequent 32 weeks (to 48 weeks of age).
- As final boar testing in replacement studs is typically conducted after 24 weeks of age, a priming dose of Improvac could be given to boars at approximately 16 weeks of age, prior to undergoing final testing, without any negative impact on testicular development and future breeding potential.
- Boars that subsequently fail the selection test could then be given a second dose of Improvac, enabling them to be slaughtered free of boar taint approximately 4 weeks later.

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## Conflict of interest

Drs Oliveira, Mathur, and Allison are all employees of Zoetis. Dr Scheid is the head of Scheid Assessoria Agropecuária, the Brazilian company that conducted the research under contract with Zoetis. Dr Soncini is an employee of Scheid Assessoria Agropecuária. Dr Hennessy, an honorary Senior Fellow at the University of Melbourne, was provided a consultancy fee to review the research report from Scheid Assessoria Agropecuária and prepare this paper for publication.

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# In vitro antimicrobial susceptibility of *Mycoplasma hyorhinis* field isolates collected from swine lung specimens in Korea

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## Summary

*Mycoplasma hyorhinis* is a very common inhabitant of the respiratory tract of pigs with or without pneumonia. Because there is no vaccine available to control *M. hyorhinis*, chemotherapy is the most practical way to treat disease associated with *M. hyorhinis* infection. Therefore, we tested the antimicrobial susceptibility of *M. hyorhinis* isolates recovered from lung specimens of pigs using the liquid minimum inhibitory concentration (MIC) method in tests with 12 antimicrobial agents. The MIC<sub>50</sub>, MIC<sub>90</sub>, and range of MICs

against 10 field isolates from Korea and the reference strain (ATCC 17981) were investigated. *Mycoplasma hyorhinis* field isolates were sensitive to lincomycin and tylosin but resistant to erythromycin, spectinomycin, and streptomycin. The MIC<sub>90</sub>s for lincomycin and tylosin were 0.5 µg per mL and 1.0 µg per mL, respectively. The MIC<sub>90</sub>s for amoxicillin, erythromycin, penicillin, spectinomycin, and streptomycin were ≥ 64 µg per mL. The MIC<sub>90</sub>s for gentamicin, kanamycin, and neomycin were 4.0 µg per mL, 8.0 µg per mL and 16 µg per mL, respectively. For oxytetracycline and tetracycline, the MIC<sub>50</sub> was 4.0 µg per mL and the MIC<sub>90</sub> was 16 µg per mL. These results provide practical information for treatment of *M. hyorhinis* infection in pigs.

Keywords: swine, *Mycoplasma hyorhinis*, antimicrobial susceptibility, minimum inhibitory concentrations

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## Resumen - Susceptibilidad antimicrobiana in vitro de los aislados de campo del *Mycoplasma hyorhinis* colectados de muestras de pulmón de cerdo en Corea

El *Mycoplasma hyorhinis* es un habitante muy común del tracto respiratorio de cerdos con o sin neumonía. Debido a que no hay vacuna disponible para controlar el *M. hyorhinis*, la quimioterapia es la manera más práctica para tratar la enfermedad asociada con la infección de *M. hyorhinis*. Por tanto, pusimos a prueba la susceptibilidad antimicrobiana de los aislados del *M. hyorhinis* recuperados de las muestras de pulmón de cerdos utilizando el método de concentración mínima inhibitoria (MIC por sus siglas en inglés) de líquido en pruebas con 12 agentes antimicrobianos. Se investigaron el MIC<sub>50</sub>, MIC<sub>90</sub>, y el rango de los MICs contra 10 aislados de campo de Corea y la cepa de referencia (ATCC 17981). Los aislados de campo del *M. hyorhinis*

fueron positivos a la lincomicina y a la tilosina pero resistentes a la eritromicina, espectinomicina, y estreptomycin. El MIC<sub>90</sub>s para la lincomicina y la tilosina fueron de 0.5 µg por mL y 1.0 µg por mL, respectivamente. El MIC<sub>90</sub>s para la eritromicina, espectinomicina, amoxicilina, penicilina, y estreptomycin fueron ≥ 64 µg por mL. El MIC<sub>90</sub>s para la gentamicina, kanamicina, y neomicina fueron de 4.0 µg por mL, 8.0 µg por mL, y 16 µg por mL, respectivamente. Para oxitetraciclina y tetraciclina, el MIC<sub>50</sub> fue 4.0 µg por mL y el MIC<sub>90</sub> fue 16 µg por mL. Estos resultados proveen información práctica para el tratamiento de la infección de *M. hyorhinis* en cerdos.

## Résumé - Sensibilité antimicrobienne in vitro d'isolats de champs de *Mycoplasma hyorhinis* obtenus à partir de spécimens de poumons de porc en Corée

*Mycoplasma hyorhinis* est un habitant très fréquent du tractus respiratoire des porcs avec et sans pneumonie. Étant donné qu'il n'y a aucun vaccin disponible pour limiter *M. hyorhinis*, l'utilisation d'antimicrobien est le moyen le plus pratique pour traiter la maladie associée à l'infection par *M. hyorhinis*. Ainsi, nous avons testé la sensibilité antimicrobienne d'isolats de *M. hyorhinis* obtenus de spécimens de poumons de porcs en utilisant la méthode de concentration minimale inhibitrice (CMI) en milieu liquide avec 12 agents antimicrobiens. Les CMI<sub>50</sub> et CMI<sub>90</sub>, de même que l'étendue des CMI de 10 isolats de champs provenant de la Corée et de la souche de référence (ATCC 17981) ont été étudiées. Les isolats de champs de *M. hyorhinis* étaient sensibles à la lincomycine et le tylosin mais résistants à l'érythromycine, la spectinomycine, et la streptomycine. Les CMI<sub>90</sub> pour la lincomycine et le tylosin étaient respectivement de 0,5 µg par mL et 1,0 µg par mL. Les CMI<sub>90</sub> pour l'érythromycine, la spectinomycine, l'amoxicilline, la pénicilline, et la streptomycine étaient ≥ 64 µg par mL. Les CMI<sub>90</sub> pour la gentamicine, la kanamycine, et la néomycine étaient respectivement de 4 µg par mL, 8 µg par mL, et 16 µg par mL. Pour la tétracycline et l'oxytétracycline, la CMI<sub>50</sub> était de 4 µg par mL et la CMI<sub>90</sub> de 16 µg par mL. Ces résultats fournissent des informations pratiques pour le traitement des infections à *M. hyorhinis* chez les porcs.

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*Mycoplasma hyorhinis* is a common isolate from the upper respiratory tract and tonsils of pigs exhibiting pleuritis, peritonitis, pericarditis, polyserositis, or polyarthritis.<sup>1</sup> However, it may be isolated from swine lungs with or without pneumonia.<sup>2,3</sup> *Mycoplasma hyorhinis* is responsible for considerable economic losses through growth retardation, poor feed conversion, inflammation, immunosuppression, and increased susceptibility to other infectious swine diseases.<sup>1</sup> It may occur as a secondary agent associated with both catarrhal bronchopneumonia and interstitial pneumonia.<sup>4</sup>

Chemotherapy is the most practical way to treat disease associated with *M. hyorhinis* infection, because no vaccine is available. Several studies have been conducted using the broth dilution method to examine the antimicrobial susceptibility of *M. hyorhinis*.<sup>3-12</sup> In Korea, *M. hyorhinis* infection is gradually increasing as a secondary infection with porcine reproductive and respiratory syndrome virus, but the antimicrobial susceptibility of *M. hyorhinis* has not been thoroughly investigated. Therefore, we tested the antimicrobial susceptibility of *M. hyorhinis*

isolated from pig lung specimens using the liquid minimum inhibitory concentration (MIC) method described by Hannan.<sup>11</sup>

### Isolation and identification of *M. hyorhinis*

A total of 10 *M. hyorhinis* field isolates were tested in this study. Isolates were collected directly from diagnostic swine lung specimens submitted to the Research Unit at Green Cross Veterinary Products Co, Ltd, Yongin, Korea, during 2011 and 2012. No animal-use protocol was necessary because only laboratory specimens were used.

The lung specimens had been collected from 23 weaned pigs, 30 to 70 days old, from nine swine farms. Isolates were cultured in Friis broth and on agar plates,<sup>1</sup> then tested for purity as single colonies on agar as follows. The lung specimen was cultured in Friis broth. The multiplex polymerase chain reaction (PCR) method for *Mycoplasma hyopneumoniae* and *M. hyorhinis* was performed and samples in which *M. hyorhinis* was identified as a single band were inoculated on Friis agar. Single colonies were re-inoculated into Friis broth and PCR was again performed to identify *M. hyorhinis*.

*Mycoplasma hyorhinis* colonies were then passaged 10 times in Friis broth. It was possible to repeatedly passage only 10 isolates in Friis broth and on agar. These 10 isolates were identified using the multiplex PCR method for *M. hyopneumoniae* and *M. hyorhinis*.<sup>13</sup> *Mycoplasma hyorhinis* ATCC 17981, isolated from the nasal cavity of a pig, was used as the reference strain for comparison with field isolates.

### Antimicrobial susceptibility testing

The antimicrobial susceptibility of *M. hyorhinis* was tested by the liquid MIC method,<sup>10-12</sup> which is simple to perform and convenient for testing small numbers of isolates.<sup>11</sup> The following 12 antimicrobial agents were examined: amoxicillin, erythromycin, gentamicin, kanamycin, lincomycin, neomycin, oxytetracycline, penicillin, spectinomycin, streptomycin, tetracycline, and tylosin (Sigma-Aldrich Co, St Louis, Missouri; Table 1). The inoculum concentration of *M. hyorhinis* field isolates used in the MICs was determined by serial tenfold dilutions in Friis broth. Specifically, the lowest dilution to show a color change (red to yel-

**Table 1:** Description of the minimum inhibitory concentrations (MICs) for antimicrobial agents tested against *Mycoplasma hyorhinis* field isolates and a reference strain\*

Antimicrobial	Agent	MIC (µg/mL)			Reference strain
		Field strains (n = 10)			
Class	Agent	MIC <sub>50</sub> †	MIC <sub>90</sub> ‡	Range	Reference strain
Aminoglycoside	Gentamicin	2.0	4.0	0.5-8.0	16
	Kanamycin	2.0	8.0	1.0-8.0	32
	Neomycin	8.0	16	2.0-32	≥ 64
	Spectinomycin	≥ 64	≥ 64	4.0 to ≥ 64	4.0
	Streptomycin	≥ 64	≥ 64	≥ 64	4.0
β-lactam	Amoxicillin	≥ 64	≥ 64	≥ 64	≥ 64
	Penicillin	≥ 64	≥ 64	≥ 64	≥ 64
Lincosamide	Lincomycin	≤ 0.25	0.5	≤ 0.25-1.0	2.0
Macrolide	Erythromycin	16	≥ 64	8.0 to ≥ 64	≤ 0.25
	Tylosin	0.5	1.0	≤ 0.25-2.0	≤ 0.25
Tetracycline	Oxytetracycline	4.0	16	≤ 0.25-32	≤ 0.25
	Tetracycline	4.0	16	≤ 0.25-32	≤ 0.25

\* *Mycoplasma hyorhinis* reference strain ATCC 17981. Field strains were isolated from lung specimens submitted to the Research Unit at Green Cross Veterinary Products Co, Ltd, Yongin, Korea, during 2011 and 2012. Specimens were from 23 weaned pigs (30-70 days old) from nine swine farms.

† MIC required to inhibit growth of 50% of *M. hyorhinis* isolates.

‡ MIC required to inhibit growth of 90% of *M. hyorhinis* isolates.

low) denoted the reciprocal number of color changing units (CCU), and the inoculum standard number of organisms was  $10^3$  CCU per mL. A final volume of 100  $\mu$ L of each antimicrobial agent was prepared by serial twofold dilutions (64 to 0.25  $\mu$ g per mL) in sterile distilled water in a 96-well microplate. The same volume of isolate culture ( $10^3$  CCU per mL) was inoculated into each well of plates containing diluted antimicrobials. Each plate contained uninoculated Friis broth as a sterility control and drug-free inoculum as a growth control. Plates were sealed, incubated at 37°C for 5 to 7 days, and observed daily until color changes in the wells were complete. The value of the MIC was defined as the lowest antimicrobial concentration to inhibit color change when the growth control changed from red to yellow (Figure 1).

## Results

Table 1 shows the MIC<sub>50</sub>, MIC<sub>90</sub>, and range of MICs against the 10 *M. hyorhinis* field isolates and the reference strain. Against the field isolates, the MIC<sub>90</sub> was  $\geq 64$   $\mu$ g per mL for amoxicillin, erythromycin, penicillin, spectinomycin, and streptomycin; 1.0  $\mu$ g per mL for lincomycin; and 0.5  $\mu$ g per mL for tylosin. Against the reference strain, the MIC was  $\geq 64$   $\mu$ g per mL for amoxicillin, neomycin, and penicillin;  $\leq 0.25$   $\mu$ g per mL for erythromycin, oxytetracycline, tetracycline, and tylosin; and 2.0  $\mu$ g per mL for lincomycin.

## Discussion

In this test, 10 *M. hyorhinis* field isolates were resistant to spectinomycin and streptomycin (MIC  $\geq 64$   $\mu$ g per mL). However, Ter Laak et al<sup>6</sup> and Wu et al<sup>9</sup> reported low MIC<sub>90s</sub> for these antimicrobials against *M. hyorhinis* (4  $\mu$ g per mL and 2  $\mu$ g per mL, respectively). In this study, the MIC of spectinomycin against the reference strain was low (4  $\mu$ g per mL).

Susceptibility of the field isolates to oxytetracycline and tetracycline in this study was poor, with an MIC<sub>50</sub> of 4.0  $\mu$ g per mL and an MIC<sub>90</sub> of 16  $\mu$ g per mL for both oxytetracycline and tetracycline. Aarestrup et al,<sup>8</sup> Hannan et al,<sup>7</sup> Ter Laak et al,<sup>6</sup> and Wu et al<sup>9</sup> found MIC<sub>90s</sub> of 0.25, 1.0, 2.0, and 2.5  $\mu$ g per mL, respectively, for tetracycline.

In this study, the MIC<sub>90</sub> for erythromycin was high ( $\geq 64$   $\mu$ g per mL), in agreement with the results reported by Ter Laak et al<sup>6</sup> and Wu et al,<sup>9</sup> who found MIC<sub>90</sub> values  $\geq 16$   $\mu$ g per mL, and Kobayashi et al,<sup>5</sup> who

**Figure 1:** A 96-well microplate showing the result of minimum inhibitory concentration (MIC) testing of antimicrobials against *Mycoplasma hyorhinis* cultured in Friis broth (isolates described in Table 1). Outside rows and columns were inoculated with 100 mL of Friis broth to prevent dehydration during the culture period of 5 to 7 days. The antimicrobial dilutions were performed from left to right using only the inner 60 wells. MIC = lowest antimicrobial concentration to inhibit color change (red to yellow). A: Inhibited growth of *M. hyorhinis*; B: Uninhibited growth of *M. hyorhinis*; C: Growth control (drug-free inoculum); D: Sterility control (uninoculated Friis broth).



reported MIC<sub>90</sub> values  $\geq 100$   $\mu$ g per mL. The MIC<sub>90</sub> was 1.0  $\mu$ g per mL for tylosin and 0.5  $\mu$ g per mL for lincomycin against the field isolates, in agreement with the results of other studies.<sup>5,6,8,9</sup>

In general, *Mycoplasma* species are difficult to isolate and culture because of fastidious growth requirements and slow growth.<sup>14</sup> For this reason, the MIC test for *Mycoplasma* species is complex and difficult to study. The results of this study provide new data regarding the susceptibility of Korean *M. hyorhinis* field isolates to 12 antimicrobial agents. To the authors' knowledge, these are the first published data concerning the antimicrobial susceptibility of Korean *M. hyorhinis* isolates. Further investigations should be conducted at regular intervals to determine the MICs of antimicrobials against additional field strains. Appropriate use of antimicrobial agents after a susceptibility test is the most practical way to treat *M. hyorhinis* infection in pigs.

## Implication

The Korean field strains of *M. hyorhinis* tested in this study are sensitive to lincomycin and tylosin but resistant to erythromycin, spectinomycin, and streptomycin.

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## Conflict of interest

None reported.

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# Effect of plasma transfer on survival rates of low-birth-weight neonatal piglets

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## Summary

Plasma transfer was evaluated as a strategy to enhance survival rates of low-birth-weight piglets. Plasma administration did not significantly affect weight gain or survival rates, demonstrating that plasma transfer alone cannot be used to improve survival rates of low-birth-weight piglets.

**Keywords:** swine, piglet mortality, plasma transfer, immunoglobulin transfer, plasma therapy

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## Resumen - Efecto de la transferencia de plasma en el porcentaje de supervivencia de lechones recién nacidos de peso bajo al nacimiento

Se evaluó la transferencia de plasma como estrategia para mejorar el porcentaje de supervivencia de lechones con peso bajo al nacimiento. La administración de plasma no afectó significativamente la ganancia de peso o el porcentaje de supervivencia, demostrando que la transferencia de plasma por sí sola no puede utilizarse para mejorar los índices de supervivencia de lechones con bajo peso al nacimiento.

## Résumé - Effet du transfert de plasma sur les taux de survie de porcelets nouveau-nés de faible poids à la naissance

Le transfert de plasma fut évalué comme stratégie pour augmenter les taux de survie de porcelets de faible poids à la naissance. L'administration de plasma n'affecta pas de manière significative le gain de poids ou les taux de survie, démontrant ainsi que le seul transfert de plasma ne peut être utilisé pour améliorer les taux de survie de porcelets de faible poids à la naissance.

Neonatal piglet mortality, especially in low-birth-weight (LBW) piglets, remains a major issue in pig farming. Despite advancement in pig production management over the years, up to 24% losses of newborn piglets are encountered by pig producers.<sup>1-3</sup> Causes of piglet mortality range from crushing by the sow to disease and poor viability.<sup>4,5</sup> The pain and distress that these piglets undergo prior to death remains an important animal-welfare issue.

High demand for pork in the consumer market has caused an increase in sow productivity, with sows producing big litters selected for breeding by farmers.<sup>6</sup> A big litter size is often associated with more low-birth-weight piglets and higher rates of mortality, compared with the industry average.<sup>2,7,8</sup> Small piglets have inadequate energy stores and may also have limited access to colostrum.<sup>8-10</sup> These piglets also often have inadequate maternal antibodies to protect them against common pathogens such as

*Escherichia coli*, with higher pre-weaning mortality from infections being reported in these piglets.<sup>9,11,12</sup>

Many strategies to improve immune status as well as survival of LBW piglets have been evaluated over the years,<sup>13-16</sup> including plasma or serum transfer. Overall, studies involving plasma or serum transfer in pigs<sup>15,17</sup> and other species, eg, horses,<sup>18-20</sup> have shown variable results. A pilot study undertaken at a swine-production facility (5600 sows in a continuous-flow production system), involving administration of plasma to LBW piglets, showed that pre-weaning mortality was 4% lower in the plasma-treated piglets (unpublished data). However, as the sample size was small, the results of the pilot study were not statistically significant. The current study was performed at the same swine-production facility to determine if plasma transfer could be used effectively in commercial farms as a strategy to improve the overall health of LBW piglets and reduce mortality.

## Materials and methods

This study was approved by the Charles Sturt University Animal Care and Ethics Committee and the swine production facility's animal ethics committee.

A total of 612 piglets (body weight 0.8 to 1.3 kg) from 212 dams (Large White × Landrace) were randomly allocated in equal numbers to one control and two treatment groups (piglet ID numbers drawn from a container). Piglets in this weight range were chosen on the basis of data obtained from a pilot study showing that approximately 40% preweaning mortality occurred in these piglets (unpublished data).

Fostering is standard practice in Australian pig farms to help manage big litters; therefore, fostering was undertaken in keeping with standard practice on the study farm. A total of 68 foster sows were used in this study. Not all piglets assigned to these foster sows were enrolled in the study. Piglets were fostered 6 to 24 hours after birth to a foster sow and remained with that dam for the duration of the study. The litter size per foster dam ranged from nine to 14 (average approximately 11 piglets), with the number of piglets per foster dam dependant on teat availability. The birth dams and foster dams were of parities two to four: information on parity of individual sows was not made available to the researchers.

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The following management practices were followed on the study farm, which comprised 5600 sows in a continuous-flow production system. Sows were group housed until 110 days of gestation, when they were housed singly in farrowing crates. As this study was conducted on a commercial farm, data on pre-weaning mortality, total births, and other production parameters were not available to the researchers. All piglets and pigs were housed in a conventional, naturally ventilated barn thermostatically controlled at 28°C. Piglets and pigs were given the standard vaccinations used on the study farm, fed age-appropriate diets, and allowed unrestricted access to water. Piglets remained with the sow from birth until weaning at 28 days.

Day 0 was the day of birth and Day 1 the first day of treatment. Piglets in the treatment groups received either two doses of plasma (10 mL on two separate occasions, Day 1 and Day 3) or one dose of plasma (10 mL on one occasion, Day 1) by intramuscular injection in four sites on the neck and hind legs, ie, 2.5 mL per site. On Day 1 and Day 3, control piglets received intramuscular injections of 10 mL of Hartmann's solution (also known as compound sodium lactate and similar to lactated Ringer's solution) as described for the two treatment groups.

Plasma was obtained from Large White × Landrace donor sows from the same farm and processed by ACE Laboratory services, Bendigo, Australia, using a standard commercial membrane filtration method, following guidelines approved under the Australian Pesticides and Veterinary Medicines Authority. Briefly, plasma was centrifuged and then filtered using a pressurized filtration method through three filters (5 to 0.2 microns). Immunoglobulin G (IgG) concentrations in the pooled plasma before and after processing (46.3 mg per mL and 32.4 mg per mL, respectively) were measured using a commercially available kit (Pig IgG ELISA Quantitation Set; Bethyl Laboratories, Montgomery, Texas).

Piglets were physically examined to assess their condition and weighed on Days 7, 14, and 21. Blood samples were obtained from piglets on Days 0, 2, and 6, and ELISA for detection of porcine IgG was performed on serum using a kit (Pig IgG ELISA Quantitation Set; Bethyl Laboratories). The ELISA kit was validated for quantification of porcine IgG using immunoglobulin standards supplied by the manufacturer. Mortality in

the three groups was recorded by one of the researchers daily after piglets were enrolled in the study.

All data were analysed using a linear mixed model in ASReml-R (VSN International, Hemel Hempstead, United Kingdom). This analysis was undertaken to account for variability caused by the effects of various factors (eg, foster sow and birth sow). In order to determine the effect of plasma treatment on IgG concentrations in piglets over time, a linear mixed model using restricted maximum likelihood was used. Birth weight, day of treatment, and treatment type were included as fixed terms in the model. Foster sow and birth sow were fitted as random terms. The effect of the plasma treatment on weekly weight gain was analysed using a linear mixed model with birth weight, treatment type, and day of treatment included as fixed terms. Foster sow and birth sow were fitted as random terms. Effect of plasma treatment on total weight gain was analysed with a linear mixed model with birth weight and treatment type included as fixed terms. Foster sow and birth sow were fitted as random terms. Differences were considered significant at  $P < .05$ .

## Results

The plasma administered in this study, when processed, had an estimated 30% loss of total immunoglobulins. The IgG concentration in the serum of the piglets was in the range of 17.1 to 21.6 mg per mL when measured at Day 2, and slowly declined to 14.1 to 18.4 mg per mL by Day 6 in both treatment groups and in the control group (Figure 1). Serum IgG concentrations did not differ significantly between the two treatment groups and the control group.

Similarly, there was no statistically significant difference in weight gain between the two treatment groups and the control group. When piglets were recruited into the study, they were all of low birth weight, and visually there were no differences between the piglets in the three groups. The average daily weight gain was 163.5 g in the group that received one dose of plasma, 164.0 g in the group that received two doses of plasma, and 163.9 g in the control group. The analysis of individual weights of the piglets showed that total weight gain in piglets over the 3-week period was influenced by the birth weight of the piglet ( $P < .05$ ), with those that were heavier at birth gaining more weight over this period (data not shown). The birth sow

had an influence on the IgG concentrations in the piglet serum at all sampling points, with piglets from seven birth sows having significantly higher IgG concentrations than the others ( $P < .05$ ).

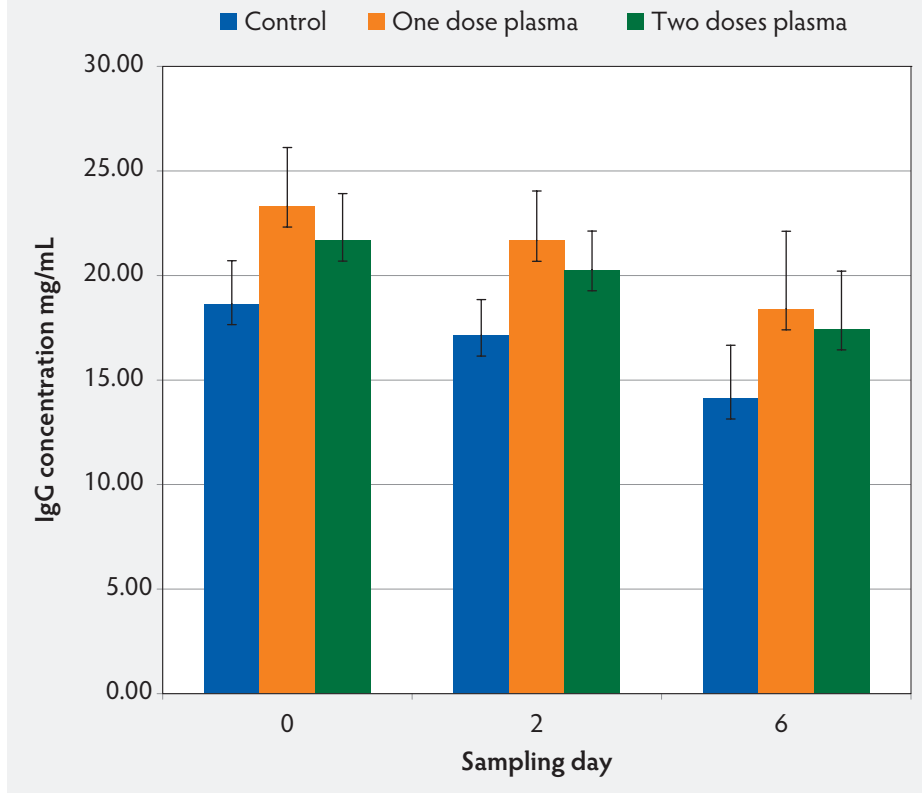
The highest mortality in piglets was recorded in those that received two doses of plasma, with most deaths occurring on Days 0, 1, and 2. By Day 21, 32.5% of piglets that received two doses of plasma, 26.6% of piglets that received one dose of plasma, and 26.5% of control piglets had died. The causes of death were poor vitality (41%), crushing by the sow (27%), and diarrhea (22%). Diarrhea was diagnosed by observation of watery stools; the causative organism of the condition was not determined.

## Discussion

The results of this study showed that administration of porcine plasma did not significantly affect LBW piglet weight gain or survival. Piglets that were heavier at birth were more robust and demonstrated better growth performance than piglets of lower birth weight. Similar results have been reported by other investigators.<sup>2,7</sup> The birth sow was an important factor in determining IgG concentrations in the serum of piglets. As IgG is the predominant immunoglobulin in colostrum, the amount of IgG taken up by the piglets depends upon the quantity or quality of colostrum obtained from the birth sow within 24 hours of birth, and this would be important in providing immunity from common infections.<sup>1,21</sup> Researchers have shown that the concentration of IgG in colostrum may be variable even within sows from the same unit.<sup>22</sup> Furthermore, IgG concentrations in colostrum also vary with the position of the teats.<sup>22</sup> In one report, the colostrum from cranial teats, for example, had higher IgG concentrations than colostrum from the caudal teats.<sup>23</sup> The birth sow would also have contributed to the first weekly weight gain of the piglets, as body fat reserves of newborn piglets are deposited in the last month of gestation.<sup>24</sup> These data underscore the importance of the birth sow in determining serum IgG concentrations of LBW piglets.

Highest mortality in this study occurred in the group of piglets that received two doses of plasma. The reason for this could not be determined. Another important finding of this study was that most deaths in LBW piglets (in all groups) were caused by poor vitality, possibly due to inadequate consumption of colostrum. It is possible that the full fostering practice adopted in this

**Figure 1:** Effect of plasma transfer on the survival of low-birth-weight piglets was determined. Piglets were randomly allocated into two treatment groups and one control group, with 204 piglets per group. Piglets in the treatment groups were administered plasma obtained from donor sows on the same farm (10 mL plasma intramuscularly in four sites per treatment) on Day 1 or Day 1 and Day 3, with Day 0 defined as the day of birth. The control group was similarly injected with 10 mL Hartmann's solution on days 1 and 3. Serum samples were obtained from the piglets on days 0, 2, and 6. Total IgG concentrations, measured using a commercially available kit (Pig IgG ELISA Quantitation Set; Bethyl Laboratories, Montgomery, Texas), did not differ significantly between groups ( $P > .05$ ).



study may have contributed to inadequate consumption of colostrum. Fostering is widely practised in piggeries across Australia, and the results of this study show that this practice may seriously disadvantage LBW piglets. Teat order is established by 24 hours following birth, and therefore fostering would cause increased fighting among the piglets, with smaller LBW piglets forced to suckle from non-productive teats.<sup>6</sup> It would be useful to explore the impact of birth and foster sows on plasma transfer in neonatal piglets, in particular, the effect of birth and foster-sow parity on IgG concentrations.

The membrane filtration method used to process the plasma may have contributed to the loss of immunoglobulins observed during plasma processing in this study. If plasma transfer is to be undertaken successfully, it is important to examine other technologies for processing plasma with a minimal loss

of immunoglobulins. In addition, strategies such as concentrating plasma by spray drying would enable larger amounts of antibodies to be delivered in smaller volumes to the neonatal piglets. On the basis of the results of this study, it appears plasma transfer alone is not sufficient to improve survival of LBW piglets. In future studies, it would be worthwhile to evaluate the efficacy of plasma transfer in conjunction with other farming practices, such as minimal fostering, in order to improve LBW piglet health and survival.

### Implications

- Under the conditions of this study, plasma transfer alone does not improve survival of LBW piglets
- Further studies are required to determine if plasma transfer used in conjunction with other farming practices can improve overall welfare and survival of LBW piglets.

### Acknowledgements

The authors would like to thank the staff on the farm in which this study was conducted for help with obtaining blood samples from the piglets, the staff at ACE Laboratory Services for processing the plasma used in this study, and Sharon Nielsen (Charles Sturt University) for help with the statistical analysis of data. This study was funded by the Australian Pork Cooperative Research Centres.

### Conflict of interest

None declared.

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## Pork Checkoff continues work on PEDV research, resources

While much work remains, 1 year after porcine epidemic diarrhea virus (PEDV) came to the United States, the National Pork Board has many research projects underway and has built an arsenal of information from the nearly \$2 million in Pork Checkoff funds designated to fight the costly disease. Additionally, the Pork Checkoff continues

collaboration with a number of industry players, including the National Pork Producers Council, the American Association of Swine Veterinarians, the American Feed Industry Association, the National Grain and Feed Association, the National Renderers Association, and the North American Spray Dried Blood and Plasma Protein

Producers. Private companies such as Cargill also have contributed funds to aid Checkoff's PEDV research efforts.

For more information, go to [www.pork.org/pedv](http://www.pork.org/pedv) or contact Paul Sundberg at [PSundberg@pork.org](mailto:PSundberg@pork.org) or 515-223-2764.

## Pork Checkoff updates Youth PQA Plus program

Consumers want to know how their food is produced. Through its Youth Pork Quality Assurance (PQA) Plus program, the National Pork Board is making training available to young producers so they can continue to earn the trust of consumers through transparency and training. Recent changes to Youth PQA Plus include an online training, testing, and certification option to accompany the current in-person

process. Delivered to students in the form of an engaging interactive, online learning module, the new online option allows participants to learn, test, and become certified in Youth PQA Plus. For youth age 12 and under, there is a parent log-in for security, as well.

Youth PQA Plus is one part of the pork industry's We Care initiative, which reflects the ongoing commitment to responsible farming and fosters continuous improvement.

Youth PQA Plus consists of two main elements: food safety and animal well-being training. The new online certification option for Youth PQA Plus was made available on April 15, 2014.

More information on the revised Youth PQA Plus program is available at [www.pork.org/certification](http://www.pork.org/certification). Click on the Youth PQA Plus link.

## New PEDV fact sheet available

**Transportation biosecurity recommendations for PEDV control at packing plants.** Because of the extreme ease with which porcine epidemic diarrhea virus (PEDV) spreads, it is very important that everyone does their part to prevent the spread of this costly disease. This means taking steps after the market pigs leave the farm. Pig transporters need to take their part in PEDV control seriously. Biosecurity procedures for the truck and trailer include cleaning and

disinfecting between loads taken to market. In addition, steps at the plant should be followed that help maintain a clear line of separation between the trailer and the market area (unloading chute).

To help keep trailers disinfected, consider using disinfectants that effectively inactivate PEDV. They include oxidizing agents, eg, potassium peroxymonosulfate (Virkon S; Antec Intl Ltd, Stevenage, England) or

sodium hypochlorite (bleach); sodium carbonates, eg, soda ash; lipid solvents, eg, ethyl alcohol; strong iodophors in phosphoric acid, eg, iodine; phenolic compounds, eg, I Stroke Environ (Steris Corp, Mentor, Ohio) or Tek-Trol (ABC Compounding, Morrow, Georgia); and aldehydes, eg, Synergize (Preserve Intl, Memphis, Tennessee).

Visit [www.pork.org/pedv](http://www.pork.org/pedv) for all related fact sheets.

## Pork Checkoff announces recipients of the 2014 pork industry scholarships

The National Pork Board has awarded 18 scholarships to college students around the United States as part of its strategy to develop the pork industry's human capital for the future. The scholarship winners were selected from a pool of 21 applicants on the basis of scholastic merit, leadership activities, pork-production industry involvement, and future pork-production career plans.

"Helping develop the next generation of pork professionals is one of the top issues that the Pork Checkoff has identified as critical for the industry's future," said Karen Richter, president of the National Pork Board and a producer from Montgomery, Minnesota. "Our ongoing service and obligation to producers includes ensuring that there is a sustainable source of young people

ready to take on the industry's charge of producing safe, wholesome pork in a socially responsible way."

For more information, contact Chris Hostetler at [CHostetler@pork.org](mailto:CHostetler@pork.org) or 515-223-2606.

# We Care at the barn level, materials available

Changing consumer attitudes makes it more important than ever for producers to show how much they care and what they do on their farm to produce safe and healthy food. To help achieve this, the National Pork Board has created a variety of materials to help producers demonstrate their commitment to the We Care ethical principles,

including We Care producer brochures that describe the proud heritage of pork and what producers are doing to show their commitment to doing what's right. There also are barn manager brochures that can be customized to highlight the ethical principles and how they can be integrated into a specific pork producer's operation. In addition,

durable We Care posters highlight each ethical principle and are available in English and Spanish.

To order We Care Barn Work Posters, contact the Pork Checkoff Service Center at 800-456-7675 or order online at the Pork Store at the top of the [pork.org](http://pork.org) home page.

## Pork Checkoff updates TQA program

Since 2001, the pork industry's Transport Quality Assurance (TQA) program has promoted responsible practices when handling and transporting pigs. In that time, TQA has undergone five revisions, always striving to offer the most current, science-based information on humane handling, biosecurity and proper transportation of swine. The mission of the TQA program remains unchanged: to continuously build a culture of protecting and promoting animal well-being through training and certification of animal handlers and transport personnel.

"Consumers are hungry for information on how their pork is raised – from the farm to the table," said Sherrie Webb, animal welfare director at the National Pork Board. "That

need for information is about more than what happens on the farm and extends to how that animal is safely and humanely transported from farm to market. That's why keeping current on transportation trends is so critical."

Staying current on transportation trends requires continuous evaluation and commitment. The Pork Checkoff's pioneering TQA curriculum focuses not only on safe handling, but also emerging diseases such as PEDV and biosecurity. The revised program provides a new approach to understanding basic pig behavior and body language and how it contributes to a safe and positive experience for both the pig and the handler.



"Calm pigs are easier to handle than excited, agitated pigs. Handling will be easier, and pigs less likely to become agitated and bunch together, if handlers use basic pig behavioral principles," said Webb. "An important part of effectively using pig behavior during handling procedures is learning how the pig perceives and responds to the handler in different situations and environments."

For more information, go to [www.pork.org/certification](http://www.pork.org/certification) or contact Sherrie Webb at [SWebb@pork.org](mailto:SWebb@pork.org) or 515-223-3533.



2014 AASV Foundation

# Midwest Golf Outing

Fox Ridge Golf Course – Dike, Iowa

Thursday, August 21, 2014 • 11:00 AM – 6:00 PM

The popular AASV Foundation Golf Outing is set for Thursday, August 21, at Fox Ridge Golf Club in Dike, Iowa. Golfers can expect a warm welcome at this location, as the club is owned by AASV member Dr Steve Menke, who practices in Ottumwa, Iowa. Dr Menke's son, Michael, serves as the club's general manager. Fox Ridge was recently recognized as winner of the golf club category in the Waterloo/Cedar Falls Courier's 2013 "Best of the Best" publication.

The foundation is pleased to recognize the industry sponsors who have provided generous support to keep golfers "fed and watered" during the event: **Uniferon** (dinner), **Aivlosin** (lunch), and **Harrisvaccines**

(beverages). In addition, golf-hole sponsors **Zoetis**, **Norsvin**, **Insight Wealth Group**, and **Alltech** will engage golfers with extra games and activities on the course.

Members of the AASV, industry stakeholders, and guests are invited to register a four-person team to enjoy this friendly 18-hole best-ball tournament. Individuals and couples are also welcome to register and will be assigned to a team. Golfers will test their combined skills against the challenges of the course and compete in individual contests along the way.

Golfer check-in begins at 11:00 AM the day of the event, with the driving range available for warming up with a few practice balls. The four-

person team, best-ball competition gets underway at 12:00 noon with a shotgun start. Box lunches and beverages will be supplied on-course. Following the golfing, team and individual contest winners will be recognized during a pork chop dinner.

The registration fee includes 18 holes of "best-ball" golf, cart rental, lunch, beverages, awards dinner, and prizes. Proceeds from the outing provide support for the AASV Foundation as it seeks to "ensure our future...create a legacy" for swine veterinarians. Income generated by the event helps fund foundation programs such as swine externship grants for veterinary students, travel stipends for students attending the AASV Annual Meeting, research funding, student-intern support, and heritage member videos.

For a sneak peek at the golf course, visit the Fox Ridge Web site: [www.golffoxridge.com](http://www.golffoxridge.com).

**For more information about the outing, contact AASV:**  
Tel: 515-465-5255  
Fax: 515-465-3832  
E-mail: [aasv@aasv.org](mailto:aasv@aasv.org)

## REGISTRATION FORM

Please complete, detach, and return this form with payment to the AASV Foundation by August 7, 2014

- Single registration ..... \$125.00  
(per person – includes 18 holes of golf, golf-cart rental, refreshments, box lunch, and closing dinner)
- Team registration ..... \$500.00  
(group of four – list names below)

1. \_\_\_\_\_
2. \_\_\_\_\_
3. \_\_\_\_\_
4. \_\_\_\_\_

I cannot attend, but will contribute to the AASV Foundation.

My tax-deductible donation is enclosed: \$ \_\_\_\_\_

Name \_\_\_\_\_

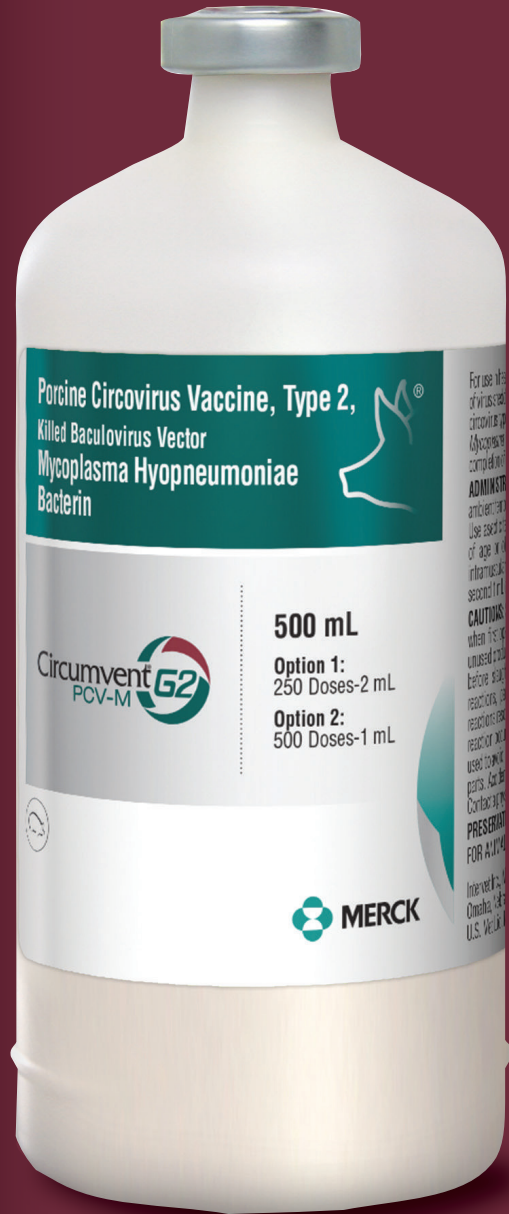
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# AASV NEWS

## Call for abstracts – Research Topics session

Plans are underway for the 46<sup>th</sup> annual meeting of the American Association of Swine Veterinarians (AASV), to take place in Orlando, Florida, on February 28-March 3, 2015. As part of the meeting, there will be a session highlighting research projects related to swine health and production. Abstracts are now being accepted for potential presentation during the Research Topics session.

Those interested in making a *15-minute* oral presentation should submit a one-page abstract on applied research related to swine health and production issues (virology, bacteriology, parasitology, environment, food

safety, odor, welfare, etc) to the American Association of Swine Veterinarians, 830 26<sup>th</sup> Street, Perry, IA 50220-2328; Fax: 515-465-3832; E-mail: [aasv@aasv.org](mailto:aasv@aasv.org).

Include the presenting author's name, mailing address, phone and fax numbers, and e-mail address with each submission. Submissions may be e-mailed, faxed, or mailed to arrive in the AASV office by **August 15, 2014**.

Abstracts not selected for oral presentation will be considered for poster presentation. All submitting authors will be notified of the selection results by October 1. Authors of

abstracts selected for oral or poster presentation must provide their paper, formatted for publication in the meeting proceedings, by November 17, 2014.

**PLEASE NOTE:** Participation in the Research Topics oral and poster session is at the presenter's expense. The presenter is required to register for the meeting (non-member participants may register at the AASV regular member rate). No speaking stipend or travel-expense reimbursement is paid by the AASV.

## Call for papers – AASV 2015 Student Seminar

### Veterinary Student Scholarships

The American Association of Swine Veterinarians announces an opportunity for veterinary students to make a scientific presentation during the Student Seminar at the AASV Annual Meeting in Orlando, Florida, on Sunday, March 1, 2015. Interested students are invited to submit a one-page abstract of a research paper, clinical case study, or literature review for consideration. The submitting student must be a current (2014-2015) student member of the AASV at the time of submission, and must not have graduated from veterinary school prior to March 1, 2015. Submissions are limited to one (1) abstract per student.

Abstracts and supplementary materials must be **received** by Dr Alex Ramirez ([alex@aasv.org](mailto:alex@aasv.org)) by **11:59 PM Central Daylight Time on Monday, September 22, 2014** (firm deadline). All material must be submitted electronically. Late abstracts will not be considered. You should receive an e-mail confirming the receipt of your submission. If you do not receive this confirmation e-mail, you must contact Dr Alex Ramirez ([alex@aasv.org](mailto:alex@aasv.org)) by Wednesday September 24,

2014, with supporting evidence that the submission was made in time; otherwise, your submission will not be considered for judging. The abstracts will be reviewed by an unbiased, professional panel consisting of a private practitioner, an academician, and an industry veterinarian. Fifteen abstracts will be selected for oral presentation in the Student Seminar at the AASV Annual Meeting. Students whose papers are selected will be notified by October 15, 2014, and will be expected to provide the complete paper or abstract, reformatted for publication, by November 17, 2014.

To help defray the costs of attending the AASV meeting, Zoetis provides a \$750 honorarium to the student presenter of each paper selected for oral presentation during the Student Seminar.

Each veterinary student whose paper is selected for oral presentation also competes for one of several veterinary student scholarships awarded through the AASV Foundation. The oral presentations will be judged to determine the amount of the scholarship awarded. Zoetis funds a \$5000 scholarship for the student whose paper, oral presentation, and supporting information are judged

best overall. Elanco Animal Health provides \$20,000 in additional funding, enabling the AASV Foundation to award \$2500 each for 2<sup>nd</sup> through 5<sup>th</sup> place, \$1500 each for 6<sup>th</sup> through 10<sup>th</sup> place, and \$500 each for 11<sup>th</sup> through 15<sup>th</sup> place.

Abstracts that are not selected for oral presentation in the Student Seminar will be considered for participation in a poster session at the annual meeting. Zoetis and the AASV fund a stipend of \$250 for each student who is selected and participates in the poster presentation. In addition, the presenters of the top 15 poster abstracts compete for awards ranging from \$200 to \$500 in the Veterinary Student Poster Competition.

Complete information for preparing and submitting abstracts is available on the AASV Web site at [www.aasv.org/annmtg/2015/studentseminar.htm](http://www.aasv.org/annmtg/2015/studentseminar.htm). Please note: the rules for submission should be followed carefully. For more information, contact the AASV office (Tel: 515-465-5255; Fax: 515-465-3832; E-mail: [aasv@aasv.org](mailto:aasv@aasv.org)).

*AASV news continued on page 207*



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Reference: 1. Buhr BL, Hurley T, Tonsor G, Zering K, DiPietro D. Comprehensive economic analysis of Improvest adoption by the US pork industry. *Am Assoc Swine Vet.* 2014;201-206.

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### Brief Summary

**CAUTION:** Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian.

**DESCRIPTION:** IMPROVEST is a sterile solution containing Gonadotropin Releasing Factor Analog-Diphtheria Toxoid Conjugate. Each mL contains 0.2 mg Gonadotropin Releasing Factor Analog-Diphtheria Toxoid Conjugate, 150 mg of diethylaminoethyl-dextran hydrochloride, 1 mg chlorocresol, sodium hydroxide as needed to adjust pH and water for injection.

**INDICATIONS FOR USE:** IMPROVEST is indicated for the temporary immunological castration (suppression of testicular function) and reduction of boar taint in intact male pigs intended for slaughter.

**DOSAGE AND ADMINISTRATION:** IMPROVEST should be administered via subcutaneous injection into the post auricular region of the neck. A safety injector should be used, preferably one which has a dual safety system providing both a needle guard and a mechanism to prevent accidental operation of the trigger. Each intact male pig should receive two 2-mL doses of IMPROVEST. The first dose should be administered no earlier than 9 weeks of age. The second dose should be administered at least 4 weeks after the first dose. Pigs should be slaughtered no earlier than 3 weeks and no later than 10 weeks after the second dose. In case of misdosing, the animal should be re-dosed immediately.

**CONTRAINDICATIONS:** Do not use IMPROVEST in intact male pigs intended for breeding because of the disruption of reproductive function. Not approved for use in female pigs and barrows.

### WARNINGS:

#### WITHDRAWAL PERIODS:

No withdrawal period is required when used according to labeling.

Not for Human Use. Keep Out of Reach of Children.

#### USER SAFETY WARNINGS:

**Warning for person administering IMPROVEST:** Accidental self injection could affect reproductive physiology of both men and women and may adversely affect pregnancy. **Pregnant women should not administer this product. Women of childbearing age should exercise extreme caution when handling this product.** Special care should be taken to avoid accidental self injection and needle stick injury when administering the product. Protective clothing including, but not limited to, safety glasses and gloves should be worn. Use a safety injector, preferably one which has a dual safety system providing both a needle guard and a mechanism to prevent accidental operation of the trigger. In case of eye contact, rinse immediately with copious amounts of water. In case of skin contact, wash immediately with soap and water. The product should be stored safely out of the reach of children. As a reminder, it is the prescribing veterinarian's responsibility to inform drug administrators of the user safety warnings associated with IMPROVEST.

**Advice to the user in the event of accidental self injection:** In the event of accidental self injection, wash the injury thoroughly with clean running water. Seek prompt medical attention and take the package leaflet with you. Do not administer the product, and/or any other product with a similar action, in the future.

**Advice to the physician:** Accidental self injection could affect reproductive physiology of both men and women and may adversely affect pregnancy. If self injection with IMPROVEST is suspected, reproductive physiology should be monitored by assay of testosterone or estrogen levels (as appropriate). The risk of a physiological effect is greater after a second or subsequent accidental injection than after a first injection. The patient should be advised not to administer IMPROVEST, and/or any other product with a similar action, in the future.

For customer service, to report suspected adverse reactions or to obtain a copy of the Material Safety Data Sheet (MSDS) call 1-888-963-8471.

**PRECAUTIONS:** Subcutaneous injection in intact male pigs can cause a transient local injection site reaction that may result in trim loss at slaughter.

**ADVERSE REACTIONS:** The field study observations from field effectiveness studies were consistent with the observations made during the target animal safety studies of transient inflammation at the injection sites. IMPROVEST did not cause unusual clinical signs or an unexpected frequency or severity of injection site reactions. Adverse events, as reported, were not uniquely attributable to IMPROVEST.

**STORAGE INFORMATION:** Store under refrigeration at 2°-8°C (36°-46°F). Once broached, product may be stored under refrigeration for 28 days. Store bottles in carton until used. Protect from light. Protect from freezing.

**HOW SUPPLIED:** IMPROVEST is available in the following package sizes:  
20 mL bottle, 100 mL bottle, 250 mL bottle, 500 mL bottle.

Revised: January 2013

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## Call for submissions – Industrial Partners

The American Association of Swine Veterinarians invites submissions for the Industrial Partners portion of the 46<sup>th</sup> AASV Annual Meeting, to be held February 28-March 3, 2015, in Orlando, Florida. This is an opportunity for commercial companies to make brief presentations of a technical, educational nature to members of the AASV.

As in the past, the oral sessions will consist of a series of 15-minute presentations scheduled from 1:00 to 5:00 PM on Sunday, March 1. A poster session will take place on the same day. Poster authors will be required to be stationed with their posters from 12:00 noon until 1:00 PM, and the posters will remain on display throughout the afternoon and the following day for viewing by meeting attendees.

Restricted program space necessitates a limit on the number of presentations per company. Companies that are members of the *Journal of Swine Health and Production* Industry Support Council (listed on the back cover of each issue of the journal) may submit two topics for oral presentation. Sponsors of the AASV e-Letter may submit an additional topic for oral presentation. All other companies may submit one topic for oral presentation. Each company may also submit one topic for poster presentation (poster topics may not duplicate oral

presentations). All topics must represent information not previously presented at the AASV Annual Meeting or published in the meeting proceedings.

Topic titles, a brief description of the presentation content, and presenter information (name, address, telephone and fax numbers, e-mail address) must be received in the AASV office by October 1, 2014. Please identify whether the submission is intended for oral or poster presentation. Send submissions via mail, fax, or e-mail to Commercial Sessions, AASV, 830 26<sup>th</sup> Street, Perry, IA 50220-2328; Fax: 515-465-3832; E-mail: [aasv@aasv.org](mailto:aasv@aasv.org).

Authors will be notified of their acceptance by October 15, 2014, and must submit the paper for publication in the meeting proceedings by November 17, 2014. All presentations – oral and poster – will be published in the proceedings of the meeting. Papers for poster presentations are limited to one page of text plus one table or figure. Papers for oral presentations may be up to five pages in length (including tables and figures), when formatted according to the guidelines provided to authors upon acceptance of their presentations. Companies failing to submit papers in a timely manner will not be eligible for future participation in these sessions.

## Interstate movement restrictions

A number of states have enacted additional movement restrictions on the importation of swine, based on their exposure to porcine epidemic diarrhea virus (PEDV). To date, we have received notification from Arkansas, Idaho, North Dakota, Oklahoma, Texas, Utah, and Washington requiring permits, certificates of veterinary inspection, or additional statements of declaration regarding the PEDV status of the herd

of origin prior to shipping the animals. These changes are posted on the AASV website (<http://www.aasv.org/pedv/StateImportPEDRequirements140507.pdf>). While we make every effort to maintain the accuracy of this information and keep the list updated, always verify the import requirements with the state animal-health official before shipping the animals.

## Pain mitigation

The interest in responding to concerns about finding a way to provide swine farmers with a mechanism to mitigate pain associated with castration and tail docking has led veterinarians to consider the use of analgesics. The discussion has revolved around the lack of approved analgesic or anesthetic products for use in swine. Lidocaine is one product that has been considered. It is our opinion at AASV that, although not approved for use in swine, lidocaine is approved by the US Food and Drug Administration (FDA), and therefore could be used in an extra-label manner if the conditions of the Animal Medicinal Drug Use Clarification Act (AMDUCA) can be met. AMDUCA requires the existence of a valid Veterinary-Client-Patient Relationship; extra-label use limited to circumstances when a threat is posed to the health of an animal or when failure to treat results in suffering

or death; the lack of an approved product to address the issue; the availability of an approved animal or human drug that would address the issue; the product be administered on the direction of a licensed veterinarian, appropriately labeled and assigned an extended withdrawal time to avoid violative residues.

We received a letter from the FDA confirming that the extra-label use of FDA-approved drugs is acceptable under the regulations set forth in AMDUCA to alleviate pain associated with surgical procedures such as castration and tail docking. This letter was in response to a request to the agency from Dr Liz Wagstrom, Chief Veterinarian, National Pork Producers Council.

In addition, Dr Craig Lewis from FDA gave a presentation during the Animal

Care Committee meeting at the National Institute for Animal Agriculture Annual meeting on the legal use of drugs in food animals for pain mitigation. We have posted his presentation on the AASV Web site for your reference. Dr Lewis identifies common products associated with pain mitigation and discusses whether or not they could be used legally for pain control in food-producing animals.

Lidocaine is commonly suggested as a drug that possibly could be used in an extra-label manner to alleviate pain resulting from surgical procedures in food-producing animals. The FDA agrees, but notes that lidocaine is NOT approved for veterinary use. Therefore, only the lidocaine approved for human use can legally be prescribed under AMDUCA for extra-label use in food animals.

## FDA announces voluntary withdrawal of 16 antimicrobials for use in food-producing animals

The US Food and Drug Administration (FDA) has announced that five drug sponsors holding animal-drug applications affected by Guidance For Industry (GFI) #213 have requested that FDA withdraw approval of a collective 19 animal-drug applications because the products are no

longer manufactured or marketed. Of these 19 applications, 16 are antimicrobials affected by GFI #213. The guidance outlines FDA's plan to help curb antimicrobial resistance by, among other things, phasing out the use of medically important antimicrobials in food-producing animals

for production purposes. A complete list of products and manufacturers is posted on the agency's Web site. Source: [http://www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm392461.htm?source=govdelivery&utm\\_medium=email&utm\\_source=govdelivery](http://www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm392461.htm?source=govdelivery&utm_medium=email&utm_source=govdelivery).

## AASV Practice Tips now online

Each year at the AASV Annual Meeting, the Practice Tips pre-conference seminar shares useful information to benefit the "boots-in-the-barn" veterinarian. While there are no formal proceedings papers for these practice tips, the participants have made their presentation slides available as part of the Swine Information Library. If your membership in the AASV is current, you can access them here: [https://www.aasv.org/library/swineinfo/series\\_index.php?id=12#106](https://www.aasv.org/library/swineinfo/series_index.php?id=12#106). Presentations include the following:

- You want me to do what?? The crazy world known as the show pig industry! – Amy Woods
- Motivating employees through purpose discovery – Larry Coleman
- More than just a tip – Joshua Barker
- Shipping samples: Regulations and compliance inspections – Melissa Hensch

- Investigations, illusions and integrity: What we learned from an FDA audit – Carissa Odland (2nd place)
- Tracheobronchial mucus collection: A novel way for herd detection of *Mycoplasma hyopneumoniae* – Kimberly Crawford (3rd place)
- Communicating to incite action – Aaron Lower
- Feedback tips and tricks – Rebecca Robbins
- Let's take another look at that pig – Joe Rudolphi
- A veterinarian's options for inactivating PEDV in hog trailers – Paul Thomas
- Ovugel use in timed breeding – Todd Price
- PEDV survival 101 – Matthew Turner (1st place)

Thanks very much to Dr Jay Miller, who organized and chaired the seminar, and to the presenters for their willingness to share their knowledge and experience with their AASV colleagues, both at the meeting and online.



# FOUNDAATION NEWS

## AASV Foundation establishes Legacy Fund, reopens Lemman Fellow program

In an effort to improve the AASV Foundation's effectiveness in fulfilling its mission, the foundation board has set its sights on increasing its endowment. To accomplish this goal, a new giving program, the Legacy Fund, joins the Heritage and newly re-opened Lemman Fellow programs to provide a trio of opportunities for contributing to the endowment.

The endowment provides the financial footing that enables the foundation to sustain its support for research, scholarships, and other projects well into the future. Endowed contributions, including all donations to the Lemman and Heritage Fellow programs, are invested to generate income in the form of interest, dividends, and capital gains. The income is used to fund foundation activities, while the original contribution is conserved, helping to assure the organization's long-term stability and success.

The new **Legacy Fund** provides an opportunity to recognize a principal donor – or an honoree – through a significant contribution to the endowment. A donor (or multiple donors) may establish and name a Legacy Fund with a gift of \$50,000 or more.

The fund may be named after the donor or another individual or group. Additionally, the donor designates which of three foundation mission categories the fund proceeds will support: research, education, or long-range issues. The board anticipates that AASV members will appreciate the opportunity to join together to provide lasting support to the foundation in honor of a mentor or in recognition of a shared experience such as the Executive Veterinary Program or the AASV presidency.

In addition to establishing the Legacy Fund, the foundation board has re-opened the **Lemman Fellow** program to allow additional members to join this prestigious group. Initiated in 1995 and named for the late industry leader and former AASV president Dr Allen D Lemman, the program is responsible for the original creation and growth of the endowment, focusing on contributions from individuals. It recognizes contributors of \$1000 or more with the title of "Lemman Fellow." The foundation currently boasts 121 Lemman Fellows, recognized at <https://www.aasv.org/foundation/leman.htm>.

The **Heritage Fellow** program completes the triad of endowment giving options, recognizing contributions of \$5000 or more. While the Legacy and Lemman programs are based upon monetary donations, the Heritage Fellow program offers additional contribution options, including life-insurance policies, estate bequests, and retirement-plan assets. Heritage Fellows receive a plaque and lapel pin when they are recognized during the foundation's annual luncheon. Since the program's inception in 2001, the roster of Heritage Fellows has grown to 43 members, identified at <https://www.aasv.org/foundation/heritage.htm>.

For more information about the AASV endowment giving programs, or to make a contribution, see <https://www.aasv.org/foundation> or contact the AASV Foundation: Tel: 515-465-5255; E-mail: [aasv@aasv.org](mailto:aasv@aasv.org).



## Scientific reality in a political world

It's a fast-paced world out there. Going to the library took too long, so Al Gore invented the internet. Snail mail was too slow, so we moved to e-mail. People could drone on incessantly on e-mail, so we started texting and limited people to 160 characters. And now we're way too busy to read a text, so I'll just send you photo with a caption that disintegrates 10 seconds after you view it. I guess it's not surprising that this desire for brevity has crept into our decision-making process as well. Science is slow. We all know the world is flat, right? Why bother to prove it? When you're trying to make a point, why worry about a denominator or the benefit side of a risk assessment?

It's concerning enough when I see marketing campaigns built around half-truths and misleading tag lines, but it stops being funny when our government agencies start doing it. The answer to controlling the spread of porcine epidemic diarrhea virus (PEDV) doesn't lie in mandatory reporting and regulatory movement controls. The end to antimicrobial resistance won't come by removing growth-promotion uses of drugs or imposing additional antimicrobial-use restrictions based on the precautionary principle. Those are poorly disguised political responses to squeaky wheels.

The adverse consequences of such decisions can be significantly harmful. While

mandatory reporting of emerging diseases is probably a good thing, poorly considered government regulation can lead to increased distrust and concerns over confidentiality of information provided to the government under threat of unnecessary restrictions on a farmer's or veterinarian's livelihood. At the time I'm writing this article, Secretary Vilsack and the United States Department of Agriculture (USDA) continue to consider how they will carry out the secretary's charge for mandatory reporting of PEDV cases. It is my hope that they will move forward in a thoughtful manner, engaging all impacted stakeholders to arrive at a judicious, well-considered plan that supports the needs of the pork industry and doesn't just fill a database with useless information and minds with distrust. The industry has proposed suggestions to promote disease reporting and sharing of necessary data. The USDA needs to continue to work with industry in a cooperative manner to arrive at a solution that supports the industry while providing the agency with the information it needs to do its job.

We'd been told PEDV was a severe disease and high risk for introduction into the US swine herd. Many of us had learned about porcine epidemic diarrhea for ourselves while visiting or working in China or interacting with Chinese veterinarians and producers. We chose to ignore the warnings. We should have exercised the scenario defining who plays what roles in the event

of introduction of a non-regulatory severe production disease. We need to find a way to share data with state and federal officials that protects business interests and allows us to utilize the tools USDA has to offer. Also, maybe least cost isn't always best practice.

On the antimicrobial front, we need to ensure that we are following the regulations regarding antimicrobial use, extra-label drug use, and compounding, whether we agree with them or not. We have to police ourselves first. We cannot tolerate injudicious or illegal product use, particularly given that our clients are producing food for human consumption. Part of my job is answering the hard questions from legislators, regulators, media, our colleagues, and others regarding how we use antimicrobials in swine. We have to be able to stand up and confirm that additional veterinary oversight of antimicrobial use will ensure judicious use.

So yes, it's a fast-paced world out there. But should we allow that desire for speed and brevity to justify a move away from the diligence afforded us by adherence to the scientific principle and allow politics to rule the day? To paraphrase that "so-last-century" TV game show "Name That Tune," "I can answer that question in one note." "No." Let's not forget to take the time to include both sides of the equation. It's the denominator that provides the perspective imperative to any judicious decision.



# UPCOMING MEETINGS

## 24<sup>th</sup> Annual Swine Health and Production Conference

September 9, 2014 (Tue)  
Western Illinois University Union, Macomb, Illinois

Hosted by Carthage Veterinary Service, Ltd

For more information:

Karen Jacquot, Training and Education Coordinator  
PO Box 220, Carthage, IL 62321

Tel: 217-357-2811; Fax: 217-357-6665

E-mail: [kjacquot@hogvet.com](mailto:kjacquot@hogvet.com)

Web: <http://www.hogvet.com/conf-overview.htm>

## Allen D. Leman Swine Conference

September 13-16, 2014 (Sat-Tue)

St Paul RiverCentre, St Paul, Minnesota

For more information:

Veterinary Continuing Education  
1365 Gortner Ave, 462 Veterinary Medical Center  
St Paul, MN 55108

Tel: 800-380-8636 or 612-624-3434; Fax: 612-625-5755

E-mail: [vmedce@umn.edu](mailto:vmedce@umn.edu)

Web: <http://www.LemanSwineConference.org>

## 2014 USAHA and AAVLD Joint Annual Meeting

October 16-22, 2014 (Thu-Wed)

Sheraton Kansas City at Crown Center, Kansas City, Missouri

Hosted by United States Animal Health Association (USAHA)  
and American Association of Veterinary Laboratory Diagnosticians  
(AAVLD)

For more information:

Web: <http://www.usaha.org/Home.aspx>

## 2014 Leman China Swine Conference

October 20-22, 2014 (Mon-Wed)

Qujiang International Conference Center, Xi'an, China

Organized by the University of Minnesota

For more information (China):

Shixin and Lamp International Exhibition (Beijing) Co, Ltd  
Room 919, Qinghe Qiangyou Building  
Haidian District, Beijing, China 100085

Tel: +86 10 62928860; Fax: +86 10 62957691

E-mail: [cisile@12b.com](mailto:cisile@12b.com)

Web: <http://www.shixinlamp.com>

For more information (United States):

Dr Bob Morrison

Tel: 612-625-9276

E-mail: [bobm@umn.edu](mailto:bobm@umn.edu)

Web: <http://www.cvm.umn.edu/lemanchina/>

## Swine Disease Conference for Swine Practitioners

November 13-14, 2014 (Thu-Fri)  
Ames, Iowa

Hosted by Iowa State University

For more information:

Conference Planning and Management  
Iowa State University

1601 Golden Aspen Drive #110, Ames, IA 50010

Tel: 515-294-6222; Fax: 515-294-6223

E-mail: [registrations@iastate.edu](mailto:registrations@iastate.edu)

## 2014 North American PRRS Symposium and PED Update

December 5-6, 2014 (Fri-Sat)

Intercontinental Chicago Magnificent Mile  
505 N Michigan Ave, Chicago, Illinois

For more information:

Megan Kilgore

Kansas State University

Tel: 785-532-4528

E-mail: [vmce@vet.k-state.edu](mailto:vmce@vet.k-state.edu)

## American Association of Swine Veterinarians 46<sup>th</sup> Annual Meeting

February 28-March 3, 2015 (Sat-Tue)

Buena Vista Palace Hotel and Spa, Orlando, Florida

For more information:

American Association of Swine Veterinarians

830 26th Street, Perry, IA 50220-2328

Tel: 515-465-5255; Fax: 515-465-3832

E-mail: [aasv@aasv.org](mailto:aasv@aasv.org)

Web: <http://www.aasv.org/annmtg>



For additional information on upcoming meetings: <https://www.aasv.org/meetings/>



**American Association of Swine Veterinarians**  
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## Photo Corner



Weaned nursery pigs in Shaanxi Province, China

*Photo courtesy of Dr Aaron J. Lower*

## AASV Industry Support Council

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